VOLUME 4 November 1955 NUMBER 6

THE AMERICAN JOURNAL OF Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

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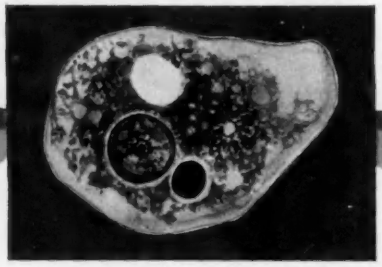

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1. Lillie, R. D., Histopathologic Technic 1948, pp. 157-160.
2. Chiffelle and Putt, Stain Technology, 26:51, 1951.

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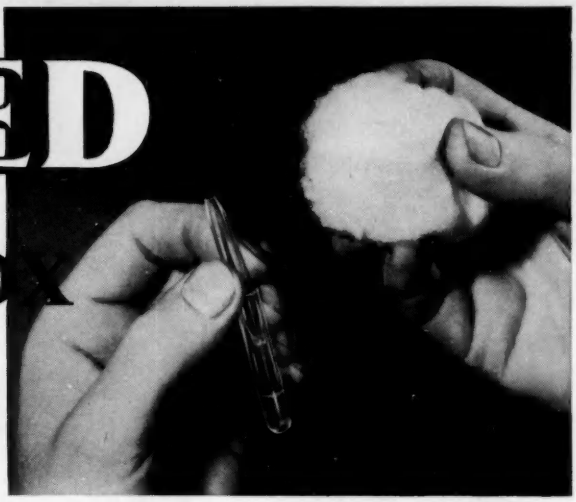

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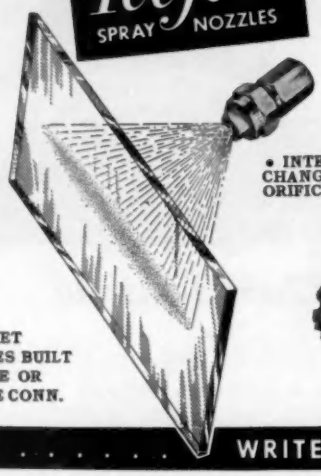

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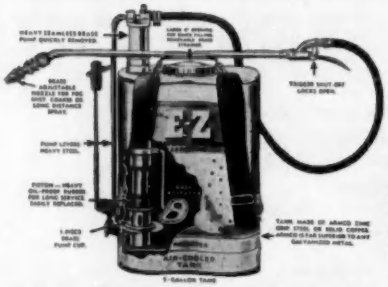

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J. ANTONIO MUÑOZ, CARLOS PÉREZ AND NEVIN S. SCRIMSHAW

Dirección General de Sanidad Pública and Instituto de Nutrición de Centro América y Panamá (INCAP)[1], *Guatemala, C.A.*

It has become increasingly clear that endemic goiter, while not specifically a tropical disease, is a very common contributor to the frequently suboptimal health of persons living in tropical and subtropical areas. The extensive survey program stimulated by the World Health Organization and its Regional Office for the Americas, the Pan American Sanitary Bureau, has shown every country on the mainland of the American tropics to have areas in which endemic goiter is a serious public health problem (Scrimshaw, 1954).

In Guatemala endemic goiter has been recognized for many years. Guerrero (1908) described a goitrous zone in the south of the country and Díaz (1918) presented further evidence as to the occurrence of goiter in Guatemala. A questionnaire sent to the mayors of rural villages by Herrera (1939) revealed at least seven goitrous zones. In 1950 systematic surveys were begun by the Institute of Nutrition of Central America and Panama (INCAP) in cooperation with the nutrition field unit of the national health department. Progress reports have been published locally (Muñoz, 1951; Pérez, 1951). The present summary report includes all of the data obtained from the cooperative studies.

Approximately 1.4 per cent of the estimated total Guatemalan population of 2,788,122 were examined. The extent and severity of endemic goiter in Guatemala, as revealed by these surveys, was so great that in November, 1954, legislation requiring the iodization of all salt destined for human consumption was approved.

MATERIAL AND METHODS

A total of 39,484 persons from all 22 departments of the country were examined, of whom 70 per cent were school children, 2 per cent pre-school children and 28 per cent adults. In eight of the departments only the capital was visited but in the others the survey included both the capital city and a number of other towns and villages.

The technique of the examination for goiter was that recently recommended by the Third Latin American Conference on Nutrition (1953). The thyroid area was first inspected with the head thrown backward and then palpated with the head forward and the neck muscles relaxed. Only glands considered by palpation to be four to five times normal size or more were classified as positive for goiter. Normal size of a lateral lobe was taken to be approximately that of the thumb nail of the person being examined. For the purposes of the survey any gland of

[1] A cooperative Institute for the study of human nutrition, supported by the governments of Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama and administered by the Pan American Sanitary Bureau, Regional Office of the World Health Organization. INCAP Scientific Publication I-48.

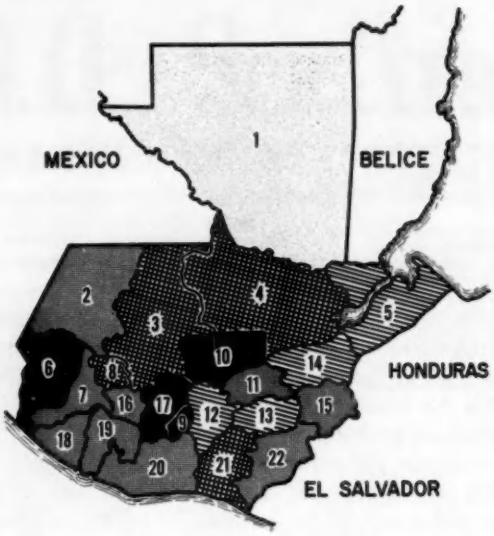

FIG. 1. Endemic goiter in Guatemala

No.	Department	Population	No. Examined	% Positive
1	Petén	15,897	659	7
2	Huehuetenango	198,872	2,283	37
3	Quiché	174,882	826	44
4	Alta Verapaz	188,758	1,550	42
5	Izabal	55,191	1,091	20
6	San Marcos	230,039	1,166	54
7	Quezaltenango	183,588	2,172	30
8	Totonicapán	99,434	592	45
9	Sacatepéquez	59,975	6,216	56
10	Baja Verapaz	66,432	1,068	64
11	Progreso	47,678	1,338	30
12	Guatemala	441,085	6,948	28
13	Jalapa	75,091	800	22
14	Zacapa	69,533	944	22
15	Chiquimula	112,837	1,021	33
16	Sololá	82,869	834	37
17	Chimaltenango	122,310	2,371	59
18	Retalhuleu	66,066	756	38
19	Suchitepéquez	125,196	1,353	37
20	Escuintla	123,809	3,487	34
21	Santa Rosa	109,812	1,352	39
22	Jutiapa	138,768	657	36
		2,788,122	39,484	38

doubtful or borderline size was classified as normal. Glands which were palpable as four to five times enlarged but smaller than those in size two were listed as size 1. Markedly enlarged glands easily visible with the head extended were classified as size 2 and those visible at a distance of several meters with the head in normal position as size 3. Adenomatous nodules of varying size were frequent and their presence was always noted.

RESULTS

The distribution of endemic goiter in the different departments of Guatemala is shown in Figure 1, together with a legend giving the population of the department, the number of persons examined in each and the percentage of goiter encountered. Only one department, Peten, had a percentage of goiter below 10 per cent while the department of Baja Verapaz had nearly 65 per cent goiter. Of the remaining departments, three had more than 50 per cent goiter and the rest between 21 per cent and 50 per cent. By weighting the percentage found in each department for the relative population of the department, the overall average for the entire country was calculated to be 38.5 per cent.

The distribution of goiter by age and sex is shown in Table 1. The finding of an equal incidence in males and females in Guatemala is of special interest and has been apparent in the survey results from the beginning. A somewhat greater incidence of goiter prevailed among adolescents than among primary school children. No special significance should be given to the higher figure for goiter in adult men and women since adults were examined from only six departments, all of them relatively high in goiter incidence, while children were examined in every one of the departments.

The size and type of goiter encountered in each age group is tabulated in Table 2. Twenty eight per cent of the goiter in adults, 14 per cent in adolescents and

TABLE 1

Distribution of goiter by age and sex

Age	Males		Females		Total	
	No. examined	% with goiter	No. examined	% with goiter	No. examined	% with goiter
0–12	14,384	34.6	11,640	33.7	26,024	34.2
13–18	5,202	46.4	4,076	45.6	9,278	46.0
19+	2,717	56.0	1,758	54.6	4,475	55.5

TABLE 2

Type and severity of goiter

Age	No. Examined	% Diffuse Goiter			% Adenomatous Goiter		
		Size 1	Size 2	Size 3	Size 1	Size 2	Size 3
0–12	26,024	24.8	6.4	0.8	1.3	0.7	0.2
13–18	9,268	30.2	10.6	1.4	2.1	1.5	0.3
19+	4,475	19.7	9.7	2.0	8.0	9.2	6.9

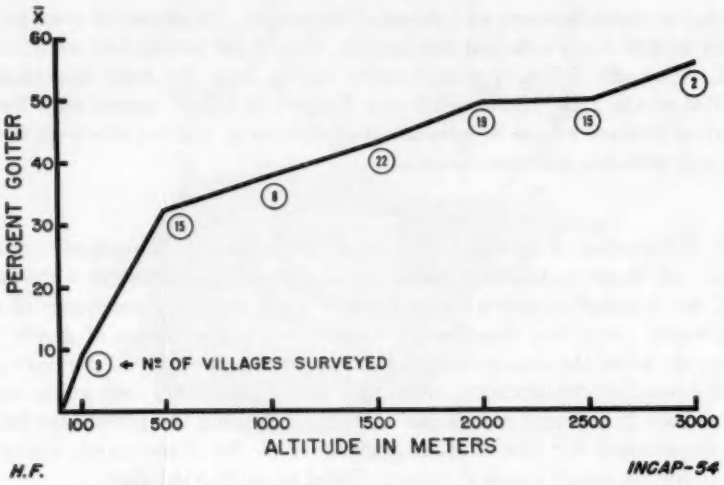

FIG. 2. Distribution of enedmic goiter in Guatemala by altitude

8 per cent in children was readily visible (size 2 or 3). Slightly over 30 per cent of the goiters identified were found to contain adenomas. These nodules were not infrequent even in the youngest children examined. Since analysis of the data did not show a significant difference between the incidence of goiter in Mestizo and Indians residing within the same locality, no separate tabulations are presented for these groups.

In those departments with sufficient localities examined to permit comparison, except for Guatemala City and El Progreso, the differences in the incidence of goiter between persons living in the capital town of a department and those living in other towns and villages were not significant. In the department of El Progreso only 4 per cent of 320 children in the capital town were found to have goiter and only one child with endemic goiter was encountered among 196 examined in five nearby villages. Nevertheless, in the same department towns with incidence rates of 30, 39, 49, 58 and 74 per cent brought the department average to 31 per cent. Moreover, the capital cities of the surrounding departments had incidence rates of 22, 22, 25 and 56 per cent. The incidence of endemic goiter in Guatemala City was 25 per cent while that of the remainder of the department was 37 per cent. It should be noted that very few persons from middle and upper income groups were included in the survey, even in Guatemala City.

In Figure 2 average values for populations living at different altitude intervals are plotted. These figures are based on 90 of the centers of population studies. The number of centers falling within each altitude range is shown within the circles. The incidence of endemic goiter in Guatemala does show a definite correlation with altitude.

DISCUSSION

Probably no other country in the hemisphere has as widespread and severe a goiter problem within its national territory. Even by the conservative criteria used for the diagnosis, over a third of the population of Guatemala suffers from this disorder. The only department with an incidence of endemic goiter of less than 20 per cent is so scarcely populated as to contain only 0.5 per cent of the population.

If Kimball (1951) is correct in his belief that adenomatous goiters have their origin in iodine deficiency in the mother and are in this sense congenital, the data give further evidence as to the severity of the problem. Other complications often stated to be associated with endemic goiter such as deaf-mutism and mental retardation in children, as well as cretinism for which the association has been definitely established, are not uncommon in Guatemala. Unfortunately, there are no data available as to their incidence and distribution.

The failure to find a greater incidence of goiter in adolescent and adult females than males is unusual but not wholly without precedent since surveys in New Zealand have given similar results (Clements, 1951). However, no explanation for this difference from the surveys in other countries of the region is apparent at the present time.

The reports from other parts of the world (World Goiter Survey, 1946) that endemic goiter is more frequent in inland and particularly highland areas seem to be borne out by the findings in Guatemala. It should not be concluded, however, that tropical lowland areas will necessarily have a low incidence of goiter. The warm tropical areas in the Guatemalan departments of Retalhuleu, Suchitepéquez, Escuintla, Santa Rosa, Jutiapa, Chiquimula, Zacapa, and Izabal all have an incidence of endemic goiter greater than 20 per cent. One of the most severe endemic goiter areas yet reported anywhere in the world is to be found in Panama in the tropical coastal provinces of Chiriquí (Arce Larreta, 1951) and Herrera (Reverte, 1954). In El Salvador the highest incidence was found in the predominately tropical lowland department of Sonsonate (Cabezas *et al.*, 1953). Preliminary INCAP surveys in the tropical areas of Nicaragua are also encountering an appreciable incidence of goiter.

It has already been demonstrated that endemic goiter, as it occurs in children in Guatemala, can be greatly reduced by the administration of iodine at the level corresponding to that obtained from a daily intake of 10 grams of salt containing 1 part of iodine in 10,000 parts of salt (Scrimshaw *et al.*, 1953).

Although a complete survey has not been done, samples of Guatemala salt as first produced have been found to have as much as .16 parts of iodine per 10,000 parts of salt when analyzed by the alternative A.O.A.C. method (Official Methods of Analysis, 1950). This is above the level of 1 part per 100,000 recommended by the WHO Study Group on Endemic Goiter (1952) for *iodized* salt. Even though part of this iodine content is rapidly lost, INCAP studies show that as much as half of it may still be present after six months. Samples purchased at random in the market in Guatemala City during February of 1954 averaged .07 parts of iodine per 10,000 parts of salt.

Since the iodine content of the local salt is so nearly that believed sufficient to prevent goiter under ordinary circumstances, it is probable that one or more goitrogenic factors are increasing the physiological need for iodine. No direct experimentation has yet been carried out in Guatemala on this aspect of the problem. However, dietary surveys have shown vitamin A deficiency to be common, and vitamin A deficiency in turn has been shown to have a goitrogenic effect (Greer, 1950). Although lime is used in the treatment of corn for the preparation of tortillas, the surveys show that the total amount of calcium thus ingested is usually below recommended dietary allowances and therefore unlikely to exert a goitrogenic effect. A search must be made for specific goitrogenic factors in the Guatemala environment.

On the basis of present information, the Third Latin American Conference on Nutrition (1953) would seem to have been well advised in suggesting a higher level of iodization for salt in Latin America than that advocated by the WHO Study Group. The final resolution of this conference took into account the variations in salt consumption, data on physiological demands for iodine, the probable action of goitrogenic factors, and the successful experience for many years of the United States and Canada in recommending that the levels should not be less than 1 part of iodine per 20,000 parts of salt and not greater than 1 part in 10,000.

Salt in most tropical countries is consumed in a crude moist form unsuitable for iodization by methods employing potassium iodide. These necessitate the refining and drying of the salt, the addition of chemical stabilizers and packaging in moisture-proof containers. The resulting increase in price and change in the appearance of the salt which have been almost insuperable obstacles to its iodization in many tropical areas, have recently been overcome by the introduction of potassium iodate for this purpose. Potassium iodate has been demonstrated to be effective (Scrimshaw *et al.*, 1953; Stacpoole, 1953) and is stable for long periods of time even when added to crude salt without special refining, stabilizing or packaging. Thus salt can now be iodized and distributed in humid and tropical areas without changing habit patterns or increasing the price to the consumer.

Although no detailed surveys have been carried out in Central America, it is well known that iodine deficiency occurs in animals and may parallel the occurrence of human goiter. For this reason salt given to animals in tropical areas with a significant incidence of endemic goiter in the human population should also contain iodine. Potassium iodate also would seem to be the most suitable compound to use for this purpose.

SUMMARY

Of a total population estimated at 2,788,122 more than 39,484 persons were examined of whom 70 per cent were school children, 2 per cent pre-school children and 28 per cent adults. The overall incidence of goiter was 38.5 per cent. Only one out of 22 departments had an incidence less than 20 per cent. One department had more than 60 per cent goiter, three between 51 and 60 per cent and

the 17 remaining departments from 21 to 50 per cent. No differences in incidence of goiter were found between males and females in any age range, between Mestizos and Indians in the same locality nor persons living in department capitals and outlying villages. The average incidence of goiter increased with altitude. Endemic goiter is considered to be a serious public health problem in Guatemala and its prophylaxis through the iodization of all salt for human consumption has been approved by the government at a level between 1 part of iodine in 10,000 parts of salt and 1 part in 15,000. Because the salt used is crude and moist and distributed without special packaging, the use of potassium iodate is planned for this purpose.

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OBSERVATIONS ON THE HEALTH OF UNITED STATES PERSONNEL LIVING IN CAIRO, EGYPT[1]

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The impact of living in North Africa on the health of United States personnel has been described in reports of disease among U. S. Army troops during World War II (Gowen, 1943; Wirts and Tallant, 1944). No description has been made, however, of the health experience to be expected by U. S. personnel, including families, who enter a community such as Cairo, Egypt, and live for extended periods in the established optimum peacetime environment.

It has been an impression of laymen and physicians in Cairo that some increase in illness occurred in U. S. personnel particularly in the early period after arrival. In view of the availability of a cooperative group of U. S. Department of State and U. S. Navy personnel, it appeared worthwhile to make such a study with the objective of finding how much and what kinds of illness might be expected by newcomers to this area.

Health environment. The climate of Cairo is generally equable, with hot summers (mean July temperature 84°F.), moderate spring and fall weather, and cool winters (mean January temperature 54°F.). The relative humidity is somewhat low, with less than 2 inches of rainfall annually. The city is located on the Nile, at the apex of the fertile agricultural delta, and has an estimated population of 2 million.

The areas of the city where U. S. personnel live are supplied with excellent water and water-borne sewage service, and streets are kept clean and free from insect breeding sites. Living quarters selected are generally modern and vermin proof, but usually are without central heat. There is, as a rule, no screening against insects.

In contrast with the sanitary urbanity of these residential areas selected by U. S. personnel, there are nearby areas with low levels of sanitation. These slums are occupied by the lower economic stratum, and communicable diseases of animals and human beings may be freely exchanged. Farther out from the city in the agricultural Nile delta, the peasants live in "villages", which are close-packed complexes of homes of mud bricks, each home consisting of two or more rooms below, in one of which the animals (goat, camel, cow, sheep, donkey or water buffalo) are housed for safekeeping. The delta is watered by irrigation canals, and in the villages these canals are commonly used for drinking water, washing and bathing. They are also frequent sites of defecation and urination by the peasants. In this milieu, well described by Weir and associates (1952), a maximum opportunity exists for the exchange of zoönoses by water, food, dust,

[1] The opinions expressed are those of the author, and do not necessarily reflect the views of the U. S. Navy.

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contact, by arthropod and molluscan vectors, between man and man, and between man and animals. Thus the villager is exposed early to many diseases, some of the more important of which are noted.

Schistosomiasis (Bilharziasis), due to *Schistosoma hematobium* and *Schistosoma mansoni*, is an important cause of morbidity and mortality, affecting virtually all of the rural population near Cairo. The snail vectors do not live outside the irrigation canal system, and the disease is not a hazard to persons who do not enter infected water. Trachoma attacks nearly 100 per cent of the rural population, and acute conjunctivitis, attributed mainly to *Koch-Weeks bacillus* and *N. gonorrhoeae*, is epidemic in children during the fly season from April to October. The serious ocular sequelae, including blindness (estimated as affecting 1 per cent of the population) are attributed to the episodes of acute conjunctivitis implanted on chronic progressive trachoma. These eye infections appear to be largely fly borne, and are most frequent in the rural population.

The enteric diseases are highly endemic. Typhoid and paratyphoid have extremely high incidence rates in poorly sanitated areas, estimated in one village by Weir and associates (1952) as 1.2 per cent per year for typhoid. The typhoid carrier rate is also high, possibly as much as four to six per cent of the general population (Weir *et al.*, 1952). There is evidence of conditioning to typhoid infection by previous exposure in the frequency of mild or asymptomatic clinical cases showing typhoid bacteremia. In 2,000 village children with fever of undetermined cause, Floyd (1952) found *Salmonella typhosi* in blood culture in 2.7 per cent and paratyphoid organisms in 1.4 per cent. The value of vaccination in protecting against typhoid is relative under this type of endemicity, recent outbreaks of typhoid having occurred among British troops in the Suez Canal Zone, whose vaccination had been fully carried out (Anderson and Richards, 1948). Bacillary dysentery due to *Shigella* of Flexner, Sonne, Boyd, Shiga and other types has an extremely high endemicity in the village population and 99 per cent of a group of village children were found by Floyd (1954) to harbor a *Shigella* at least once during a year of observation. A large number of the infections are clinically insignificant, or without symptoms, but permanent carriers of *Shigella* do not appear to be common, at least among children (Higgins *et al.*, 1954). The prevalence of the infection in the Cairo area correlates closely with fly prevalence. Meat and fish from Cairo markets were found to be commonly contaminated with *Shigella* by Floyd (1954). *Shigella* diarrhea and dysentery, locally called "Gyppy tummy" has somewhat less importance today as a cause of mortality than formerly, because of the development of effective treatment.

Amebiasis due to *Entamoeba histolytica* is highly endemic in the indigenous population, approaching 100 per cent of a village population near Cairo, studied by Lawless (1953) in repeated surveys. The infestation is commonly asymptomatic and may represent a carrier state. Ancylostomiasis is widespread affecting about 50 per cent of the fellahin in some rural areas.

Syphilis, gonorrhoea, chancroid, and lymphogranuloma venereum are said to be prevalent in the poorer economic class, but reliable prevalence data are not

available. Weir's group (1952) found 7 per cent of the population of one village to show positive Wasserman reactions.

Tuberculosis, both pulmonary and extra pulmonary, is common, with no reliable prevalence data avaialable. Weir and associates (1952) reported that 1.9 per cent of 2,098 villagers examined by X-ray showed evidence of active pulmonary tuberculosis. The local cattle are heavily infected with tuberculosis, and 95 per cent of the indigenous Cairo population are tuberculin reactors.

Skin diseases, including pyogenic infections, insect bites, fungus infections and otitis externa are common. In a study of a single village, Weir *et al.* (1952) found 50 per cent of the population infested with lice, and 77 per cent with fleas. These insects are an annoyance, and potential disease vectors as well. Malnutrition is said to be common, particularly those types due to low protein intake and avitaminoses A, B, C, and D are frequently seen. Nine per cent of males and 5 per cent of females in a village population were reported by Weir and associates (1952) to be suffering from pellagra. Brucellosis, due to *Brucella melitensis*, has been found by Killough, Magill and Said (1953) to be common in the Cairo area, and local animals are known to be heavily infected.

Several virus diseases are highly endemic in the indigenous population, and Paul and associates (1952) showed that poliomyelitis attacks village children in early years to such an extent that a high percentage of the adolescent population shows evidence of immunity. Relatively little paralytic poliomyelitis is seen in indigenes. Coxsackie virus and several other viruses, not yet fully identified, have been isolated from mosquitoes (Taylor and Hurlbut, 1953), from human blood (Taylor, Rizk, and Kader, 1953), and transmission of a Coxsackie-like virus by culicine mosquitoes has been shown (Hurlbut, 1953).

West Nile virus has been isolated in Egypt from human blood by Melnick and associates (1951) and found by Taylor (1953) to have attacked over 50 per cent of the village children over five years of age. The virus has been isolated from culicine mosquitoes by Taylor and Hurlbut (1953). West Nile fever is not reported, probably being an unrecognized fever during childhood. Q fever has been shown by Taylor and associates (1952) to have attacked over 50 per cent of the rural population over five years of age and *Coxiella burnetii* has been isolated from camel ticks (Hyalomma) (Taylor *et al.*, 1952).

Infectious hepatitis is reported to be an infrequent disease in natives of Cairo, although accurate incidence data are not available. From the extremely heavy incidence of infectious hepatitis in American and British troops in North Africa in the Desert Campaign and the relatively low apparent incidence in indigenous populations, it would appear that the local population may be highly immune. Typhus, both the endemic and epidemic variety, occurs sporadically. Filariasis due to *Wuchereria bancrofti* is endemic in the environs of Cairo. Leprosy occurs in the poorer sanitated areas. Canine rabies is enzootic, with over 100 human cases reported each year in the indigenous population.

A number of important diseases are not present now, or present only in a controlled form, and these represent a potential health threat if sanitation or vector control should break down. Cholera has not been reported since 1947,

when an estimated 25,000 cases, with approximately 50 per cent mortality, occurred in Egypt (Simmons *et al.*, 1951). Plague exists in foci in Upper Egypt, and small outbreaks have occurred in recent years. Malaria is presently held in check in the city of Cairo by control of vector mosquitoes. The disease is common in less well controlled areas, with 11,000 cases recorded from Lower Egypt and the Canal and Suez governorates in 1942 (Simmons *et al.*, 1951). Spirochaetosis in the form of relapsing fever is reported, and the louse borne type developed into an epidemic with a peak of 100,000 cases in Egypt in 1946 (Simmons *et al.*, 1951). Leptospirosis is not reported frequently, but it is likely that it is endemic and unrecognized, in view of the close rat-water-man association in the rural areas.

Smallpox vaccination is legally compulsory, but probably not completely done in rural areas. In 1944, 11,194 cases of smallpox occurred but no outbreaks have been seen in recent years. Diphtheria, meningococcus meningitis, mumps and measles are common. Dengue fever was last reported in epidemic form in 1937, when an estimated 400,000 cases occurred. Yellow fever is not reported from Egypt, but is endemic in the Anglo-Egyptian Sudan, and *Aedes aegypti*, the vector, has been infrequently reported in Egypt (Simmons *et al.*, 1951).

The above environment has been described in some detail to present disease hazards. In general, the U. S. personnel do not expose themselves to many of the mechanisms for contracting the above diseases, most of which are preventable by simple health precautions. Also, many of these diseases are aborted or suppressed by effective newer forms of treatment, and do not become severe or fatal. However, three major exposures of U. S. personnel to some of these diseases may occur. First, it is impossible to avoid travelling through slums and village areas, with exposure to the dust, to hordes of flies, and to mosquitoes and fleas.

Second, food supplies such as meat, vegetables, and fruit come from the village and pass through various insanitary handling processes. Meat is butchered and eaten the same day, inasmuch as no refrigeration is available in the average market. Milk is frequently delivered by vendors without refrigeration or pasteurization. Flies swarm unhindered over market products, and sanitation of foods in handling is virtually nonexistent.

The third, and perhaps most important, hazard to health of Americans in this environment is in the cook-servant. These are usually men, indifferently trained, and frequently not susceptible to training in proper food handling, since they do not comprehend the dangers of improper food handling. For example, this class of servant defecates in a native type toilet (a hole between two foot rests) and cleans the perineum with water by means of his hand, without tissue or other material. The likelihood of fecal contamination of food by hands following this procedure is apparent. Proper dish sterilization is not frequently practiced. The servant commonly maintains his family, and sometimes himself lives, in an unsanitated area, and exposes the employer's household to insects and communicable disease from that source.

To evaulate some of the effects of these health hazards on U. S. personnel, a health experience study was begun in April, 1953.

MATERIALS AND METHODS

A questionnaire form was completed by United States personnel who had recently arrived in Cairo. Data were obtained on the health history for two years prior to arrival in Cairo, and a daily health record of the state of health or illness in Cairo was kept by each individual. Two general qualities of disease were recorded: illness which permitted work or usual occupation, and illness requiring stoppage of work, or bed treatment. Diagnosis by either the patient or by a physician was taken to be acceptable.

The group of 57 comprised personnel of the United States Department of State, Point Four Organization, staff members of NAMRU-3, and their families. There were 30 males and 27 females, with an average age of 25.9 years, and a range of 0 (new-born) to 59 years. The occupation of the heads of family and single persons was secretarial or executive, and 14 subjects were engaged in medical research or health work. The period of study varied from 3 months to 19 months, with an average of 9.0 months; 42.7 person-years of health experience in Cairo are reported.

The study group represents only a small fraction of U. S. personnel in Cairo. No selection was made beyond that implied in the recent arrival, and willingness to cooperate in the study.

RESULTS

A comparison of the average annual number of sick days due to minor disease and due to incapacitating illness before and after arrival in Cairo is shown in Table 1. The more than four-fold increase in minor illness which occurred in Cairo was due in great part to a few communicable diseases, as shown in Table 2. Other uncommon causes of minor illness were mango allergy (4 episodes in one family), Q fever (1 episode), amebic dysentery (2 episodes), infectious hepatitis (2 episodes). Eight episodes of fever of unknown origin were recorded. There was one case of severe *meningococcus* meningitis. No significant increase in the incidence of incapacitating illness is noted in Cairo, when comparison is made with the previous history.

Because of the importance of diarrheal disease as a cause of morbidity, a comparison of incidence in 36 newcomers and in 21 "old hands" is made in Figure 1 and Figure 2. Among newcomers, a high initial attack rate is seen, with some decline after the first two months (Figure 1). The average number of subjects observed is small (25.7) during any month and the incidence of diar-

TABLE 1

Comparison of previous sick days/year with Cairo sick days/year

	Previous Sick Days/Year	Cairo Sick Days/Year
Severe illness	8.23	9.89
Minor illness	9.02	42.03
Total	17.25	51.92

TABLE 2

Leading causes of illness among 57 U.S. personnel in Cairo

Disease	Episodes Recorded
Diarrheal disease	201
Includes "bacillary dysentery", "diarrhea", "gastro enteritis" and "gyppy tummy"	
Colds	123
Includes "flu", "bronchitis", "coryza" and "upper respiratory infection"	
Sore throat	34
Includes "tonsillitis", "pharyngitis", and "strep throat"	
Skin infections	32
Includes furuncles, carbuncles, and "dermatitis"	
Eye infections	15

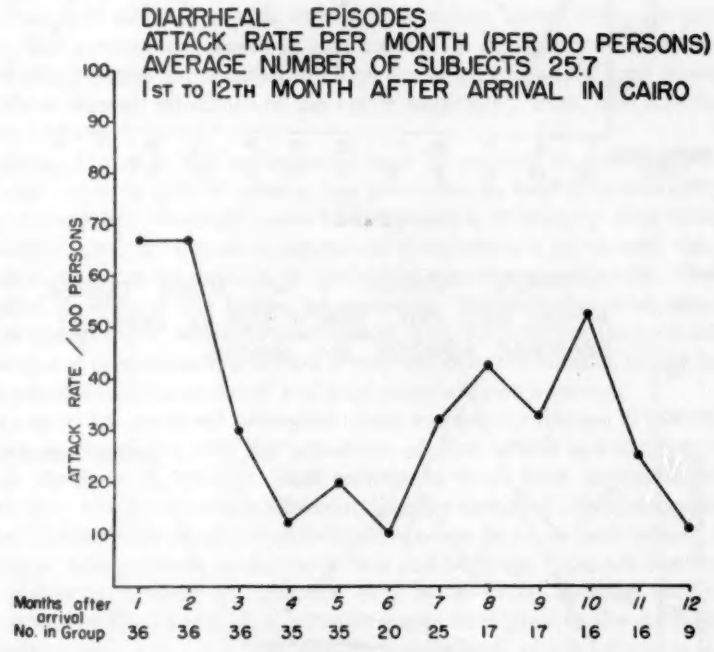

FIG. 1

rheal disease may be affected by the seasonal variation which is seen here in diarrheal disease with the increased fly prevalence from April–October. The attack rate of diarrheal disease in "old hands" (Figure 2), allowing for the small number in the group, is, surprisingly, not greatly different from the incidence curve in the newcomers.

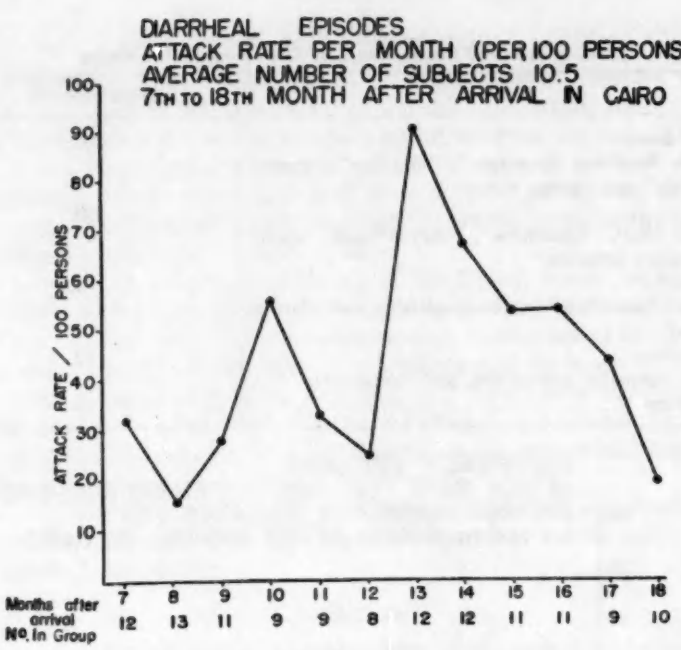
DIARRHEAL EPISODES
ATTACK RATE PER MONTH (PER 100 PERSONS)
AVERAGE NUMBER OF SUBJECTS 10.5
7TH TO 18TH MONTH AFTER ARRIVAL IN CAIRO
ATTACK RATE / 100 PERSONS
100
90
80
70
60
50
40
30
20
10
Months after arrival 7 8 9 10 11 12 13 14 15 16 17 18
No. in Group 12 13 11 9 9 8 12 12 11 11 9 10

FIG. 2

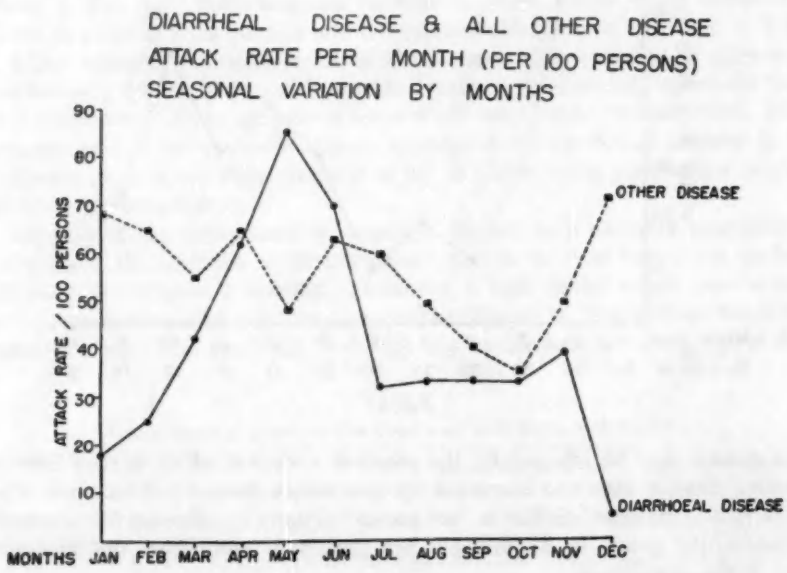
DIARRHEAL DISEASE & ALL OTHER DISEASE
ATTACK RATE PER MONTH (PER 100 PERSONS)
SEASONAL VARIATION BY MONTHS
ATTACK RATE / 100 PERSONS
90
80
70
60
50
40
30
20
10
OTHER DISEASE
DIARRHOEAL DISEASE
MONTHS JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC

FIG. 3

An incidence curve which reflects the seasonal incidence of disease in Cairo is shown in Figure 3. Diarrheal disease incidence rises sharply with the summer season, and with housefly prevalence, and drops off in winter. Conversely, other diseases reach the highest incidence in the winter months.

DISCUSSION

In the interpretation of the results of this study, allowance must be made for the small number of subjects (57), some of whom were in the study for less than a year (average 9.0 months). When the data are arranged on a person per month basis, the results are believed to be valid. Another factor must be taken in account in comparing previous health history with day to day health record: the subject may forget past minor illness and may minimize the memory of a past severe illness. Only marked differences may be taken as valid. The large proportion of medical research and trained health workers included in this study may offset this criticism somewhat.

The four-fold increase in sick days due to minor illness over previous sick days on that account appears to be significant. It is also apparent that the extra days of minor illness experienced in Cairo are largely due to a local prevalence of diarrheal disease, infections of the upper respiratory tract, and infections of the skin and eye.

Diarrheal disease in this environment may be ascribed to improper food hygiene, and occurs in spite of more or less precaution in food hygiene. A majority of the subjects ate uncooked fruits and vegetables, frequently after treatment with chemical solutions such as potassium permanganate or vinegar, the effectiveness of which as bactericidal or amebicidal agents is questionable. Flies were controlled in some of the homes by screening. Probably the most important factor in the spread of infectious diarrheas in these subjects was the cook-servant. This source of contamination of food is very difficult to eliminate, as few families find it possible to do housework and marketing without a servant.

The reason for increased infectious upper respiratory disease is not obvious, but may be associated with the prevalence of dust, which is a nuisance in the dry soil condition of the city. Dust carriage of dried feces, promiscuously deposited, may also be a factor in disseminating the agents of infectious respiratory disease. The prevalence of skin infections appears to be in part related to the ubiquity of biting insects, particularly fleas and bedbugs. Fleas are unavoidable, being picked up in theatres, taxis, and even in the home. Bedbugs require constant attention for control. Eye infections may be related to the dissemination of infectious agents by dust, although fly transmission of eye infections is probably not important in these U. S. subjects, in apparent contrast with the indigenous population. It would appear that little further improvement could be made by these subjects, in this environment, in preventing these infectious diseases of the gastrointestinal or respiratory tracts, or of the skin and eye.

Some comment may be made on the incidence and character of the diarrheal disease. In this area, a large percentage of diarrheal disease has been found to be associated with *Shigella* (Floyd, 1953). The disease was mild in these subjects,

and of unusually short average duration for each episode (3.25 days). Not much evidence of clinical immunity was apparent in comparing newcomers with "old hands" in incidence, although it has been the general opinion that newcomers to an endemic area became "seasoned" following initial attacks of shigellosis (Napier, 1946; Bulmer, 1934; Felsen, 1945).

SUMMARY

A study of illnesses among 57 Americans living in Cairo, Egypt, as compared with previous health experience, showed a four-fold increase in sick days due to minor illness. This increase was due in major part to increased incidence of infectious diarrheal disease, communicable respiratory disease, and infections of the skin and eye. The incidence and duration of attacks of diarrheal disease were not greatly lessened during the months ensuing after arrival in Cairo. The health environment with respect to communicable disease incidence in Cairo is discussed.

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INTESTINAL PARASITIC INFECTIONS IN FORSYTH COUNTY, NORTH CAROLINA

III. Amebiasis in School Children, an Index of Prevalence

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Entamoeba histolytica has become the most important protozoan parasite of man in the United States. Originally considered to be an organism largely restricted to the tropics and the sub-tropics, its distribution is now recognized as ubiquitous, determined by prevailing conditions of hygiene and sanitation.

The general prevalence has been estimated by Craig (1934) as between 5 to 10 per cent and as high as 20 per cent by Faust (1942). These figures are based upon data from two general sources: vital statistics and morbidity data, and surveys of various population groups. Neither of these sources provides data which can be translated directly into terms of actual prevalence. Reports to the United States Public Health Service and to State Departments of Health probably represent only a limited proportion of the actual cases of amebiasis (Wright, 1950). The majority of the surveys which have been reported have been conducted in the central and the southern tiers of states. In many instances these surveys have dealt with selected groups such as college students or institutional inmates rather than representative population samples. Furthermore, the data obtained from surveys in different areas are not comparable unless age-distribution analysis is included (Faust, 1936). In addition, the population samples, the methods used for the collection and examination of specimens and the criteria for specific identification of *Entamoeba histolytica* must be comparable.

EPIDEMIOLOGY

Several mechanisms for the transmission of *E. histolytica* have been postulated. These include polluted water, the use of night soil as a garden fertilizer, mechanical spread by flies and contamination of food by infected food handlers. However, until comparatively recently this protozoon has not been actually demonstrated except in association with man, flies and certain potential reservoir hosts (Ivanhoe, 1943).

It is doubtful that water plays a major role in the transfer of infection. Comparison of the prevalences of *E. histolytica* among rural families using polluted and unpolluted local water supplies has shown surprisingly little difference in the distribution of infection (Eyles *et al.*, 1953). Although water undoubtedly plays a part in regions where there is heavy contamination, water-borne outbreaks characteristically are explosive in nature and accompanied by a relatively large number of cases of diarrhoea and dysentery.

The levels of local hygiene and sanitation are important determining factors. Eyles *et al.* (1953, 1954) have shown that there is direct correlation between observed prevalences, the methods of excreta disposal and cleanliness of the domestic environment.

Incontrovertible evidence of the importance of the housefly as a vector of *E. histolytica* has been accumulated since this insect was first incriminated by Wenyon and O'Connor (1917). Cysts, viable on culture, have been recovered from the vomitus and from the droppings of flies up to twenty-four hours after an experimental feed upon infected material (Pipkin, 1942; Sieyro, 1942). Viable cysts have likewise been recovered from flies caught near latrines and in houses (Harris and Down, 1946).

The role of the food handler in the spread of infection has been controversial. An epidemiologic study of amebiasis among naval personnel was interpreted to indicate that infected food handlers are not significant sources of infection for others (Sapero and Johnson, 1939). However, a subsequent investigation of amebiasis on Aruba by Schoenleber (1940) yielded convincing evidence that the infected food handler may be an important source of spread. After several years residence, the prevalence of *E. histolytica* among foreign personnel was found to be 25 per cent and among the food handlers, 33 per cent. Improvements in hygiene, treatment of infected persons and refusal of jobs to infected individuals seeking employment as food handlers were followed in one year by a reduction of 50 per cent and, after three years, by a reduction of 92 per cent in the prevalence of infection. Craig (1944), commenting on this report, remarked: "... these observations definitely prove the great importance of the food handler in the transmission of amoebiasis and ... such a practical demonstration is worth far more than any laboratory experiment in proving that the food handlers must be controlled before we can hope to attain success in elimination of this infection."

Until recently, direct or indirect transfer of *E. histolytica* from contaminated objects other than food or drink has received little consideration. A detailed investigation of the epidemiology of amebiasis in a New Orleans orphanage provided significant evidence of this possibility (Ivanhoe, 1943). No explanation had previously been found for the persisting high prevalence of infection. Using special techniques, cysts, morphologically corresponding to those of *E. histolytica*, were recovered from the hands and soiled underwear of the children, the bottom of the laundry chute, damp sand in an outdoor play box, the contents of a wading pool and the bottom of the pool after it had been drained. Evidence in support of such possible indirect transmission was also obtained in the course of two rural studies in Tennessee (Eyles *et al.*, 1953, 1954). Their findings appeared incompatible with transmission restricted to food handlers, flies and water. In both instances direct contact with infected persons seemed best to fit the observed conditions.

Since Kofoid's (1923) original report of multiple infections by *E. histolytica* in a family, much evidence has accumulated demonstrating that amebiasis is commonly a familial disease. In 1932, Kofoid again called attention to the

family unit as an element in the epidemiology of the infection stating: "Even under the best ordered sanitary discipline there is a tendency for the infection to spread in families." The first extensive study of familial amebiasis was reported from Georgia by Seckinger (1936). The observed prevalence of *E. histolytica* among 290 persons comprising sixty-nine families was 55.9 per cent. In several families 90 per cent of the members were found to be infected. Subsequently, a report on amebiasis among children in New Orleans called attention to the fact that in almost every family concerned the infection could be demonstrated in at least one adult (Loeber and D'Antoni, 1947). These observations were amplified by D'Antoni (1952) in a report that 71 of 76 mothers and 50 of 54 fathers of infected children were likewise infected by *E. histolytica*. The significance of these findings was further emphasized by statistical analysis of multiple infections within family groups (Eyles *et al.*, 1953). This demonstrated that the number of families presenting multiple infections could not be the result of chance and that there was a high degree of correlation between the size of the family and the prevalence of infection within the domestic circle.

These same investigators found the maximum prevalence of infection among school children in the 6 to 16 year age group; adults in the household occupied an intermediate position; and the lowest observed prevalence was among preschool children 5 years of age or under (Jones *et al.*, 1954).

With the exception of the investigations of the Rockefeller Sanitary Commission prior to 1915 and a subsequent study of hookworm infection by Keller *et al.*, (1937), little information has been available concerning the prevalences of intestinal parasitic infections in the Piedmont region of North Carolina. The desirability of such information, especially concerning *E. histolytica*, was emphasized by discrepancies between reports to the North Carolina State Department of Health and findings at the North Carolina Baptist Hospital in Winston-Salem, where a prevalence of 11.5 per cent was found among 3,773 persons examined (Mackie, T. T., unpublished data). Consequently, a survey of the intestinal parasitic infections was initiated in 1950 and completed in 1952. The experimental design and statistical evaluations were carried out by the Department of Biostatistics of the School of Public Health of the University of North Carolina. The investigations were conducted by a team composed of members of the Institute of Tropical Medicine and the Department of Preventive Medicine of the Bowman Gray School of Medicine of Wake Forest College. Competent data were sought in several fields, including the general prevalence of parasitic infections in a valid sample of the population of Forsyth County (in preparation); the prevalence of amebiasis and certain aspects of the epidemiology of the infection; and the effectiveness of diiodoquin for the mass treatment of amebiasis (in press).

The various means of transmission of *E. histolytica* discussed above, particularly the potential role of the infected food handler and the possibility of indirect transfer of infection by contact with contaminated objects, and the frequency of multiple infections within the family group with the demonstration that the highest prevalence of infections occurs in the age group from 6 to 16

years, suggested that the results of examination of school children might yield information indicative of the prevalence of amebiasis in a community. This aspect of the Forsyth County Survey is the subject of this report.

STATISTICAL DESIGN

Forsyth County is situated in the central portion of the State of North Carolina. It covers an area of approximately 409 square miles and contains the highly industrialized City of Winston-Salem. The total population according to the 1950 census was 146,135, divided by race and residence as follows:

	Urban	*Rural*
White	51,051	53,642
Negro	36,760	4,682
Total	87,811	58,324

Although it was recognized that examination of stool specimens from a probability sample of the general population would be essential to avoid bias, the difficulties and expense inherent in a general screening of the population precluded this procedure. It was believed that the prevalences of the various infections would be higher among the school children than among the general population. Therefore, the decision was made to limit the survey to the school population. Bias due to lack of representation of families without children in the schools could be compensated for by statistical methods so as to permit an estimate of the County prevalence rate.

Calculations were made to determine the minimum sample size adequate to show significant differences between the racial and urban-rural groups. A maximum prevalence of approximately 15 per cent was assumed and on this basis a sample of 165 children in each race and residence group was deemed necessary. On the other hand, twice this total number of children would be required to provide a sufficient number of infections for evaluation of the treatment. The final figure was increased by 10 per cent to offset lack of cooperation and other unforeseeable difficulties which would affect the size and validity of the sample. This precaution proved extreme since cooperation exceeded 90 per cent.

Grades II, III, IV and V of the elementary school population were selected for examination. Grade I students were omitted because they were available in school only for half-day periods and they were considered too young to give full cooperation. Grade VI and older students were omitted because many individuals in this age group from poor socio-economic backgrounds may not stay in school beyond Grade V. Furthermore, there was a greater probability that these older children might be exposed to infection outside the home.

The selection of the particular schools in which the studies were to be conducted was determined by certain special considerations. Since the type of soil varies markedly between the eastern and the western portions of the County and might affect the prevalence of hookworm, one school for white children was chosen in each section. The rural Negro children all attended one consolidated school. The four urban Negro schools were quite homogeneous with respect to the composition of the student bodies and one of these schools was

sufficiently large to provide an adequate sample of the urban Negro children. Two white schools were utilized for the examination of urban white children. All four groups were representative of the middle and lower income classes and were sufficiently comparable in other respects for statistical purposes.

METHODS

Prior to the start of survey operations in each school, meetings were held with the teaching staff and the Parent-Teacher Associations at which the purposes and procedures of the survey were explained and permission slips were distributed for the signature of parents. A temporary field laboratory was set up in each school and examinations were done room by room. On the day of examination, immediately on arrival at school in the morning, the children were given crackers and a phenolphthalein-chocolate laxative.[1] This preparation was chosen because of its familiarity to the general public, its acceptability by the children, its effectiveness in producing specimens within the limited time available in the school day, and its complete freedom from toxicity and undesirable side effects. Previous field experience had demonstrated it to be the laxative of choice for survey operations of this type.

Freshly passed fecal specimens were examined immediately in the field laboratory, using saline and iodine films and Quensel's-stained preparations. Warm stages were used throughout. At the end of the school day the specimens were taken to the base laboratory for examination after concentration by the zinc sulphate centrifugal flotation method of Faust and associates (1939). Diagnoses were invariably confirmed by two or more members of the survey team and permanent-fixed preparations were made of doubtful specimens for subsequent examination after staining by the iron hematoxylin method.

The efficiency of a diagnostic technique can be calculated from the findings of successive stool examinations (Mantel, 1951). Since successive examinations of the individuals was not practical in this investigation, calculations of the diagnostic efficiency were based upon the findings at the pre- and post-treatment examinations of the individuals in the non-treated group. These indicated a probability of 59 per cent of demonstrating *E. histolytica* in an infected person by examination of one stool. This compares favorably with the 62 per cent efficiency reported by Tobie *et al.* (1951).

For the purposes of measuring the usefulness of the school child as a proband or lead to familial infection, two urban schools were utilized in the City of Winston-Salem, one for white and the other for Negro children. Apart from the racial difference, the population groups served by the two schools were comparable. Children found to be infected by *E. histolytica* in the course of the examinations in the schools and a limited number of randomly selected children not found to be infected served as the probands or leads to the "positive families" and to the "negative control families." A family was defined to include all persons living under the same roof and taking at least one meal together every day.

[1] Generously supplied by Ex-Lax Inc.

SAMPLES AND COOPERATION

The total school child sample used in the survey comprised 2,063 individuals, representing approximately one-fifth of the 10,063 children available in Grades II through V in Forsyth County. The sample included 1,071 whites and 992 Negroes; fifty-five per cent were urban residents. A total of 1,934 children submitted to examination, a cooperation rate of 93.7 per cent.

Fifty urban families, comprising 233 persons exclusive of children examined in the schools, constituted the positive group. Of these, 208 submitted to examination, a cooperation rate of 89.3 per cent.

Forty-nine urban families of apparently uninfected children, comprising 197 persons exclusive of children examined in the schools, composed the negative control group. A total of 160 of these were examined, a cooperation rate of 81.2 per cent.

The students not found infected by *E. histolytica* who served as leads to the control families were selected by systematic sampling. Every tenth negative child was chosen to assure a group of controls approximately equal to that of the positive probands. A negative student who was a sibling of a positive student was not accepted as a control proband since he would automatically be included in the family of the infected student. Examinations of the negative control families were conducted on a sequential basis until the sample reached a magnitude sufficient to provide conclusive statistical evidence of the significance of the observed difference.

The methods of examination of family members differed from those of the school children only in the elapsed time between evacuation and examination of the fecal specimens. The persons to be examined were instructed to take the laxative agent at night and to collect a stool specimen the following morning. These specimens were picked up as promptly as possible and taken to the base laboratory for examination, using the direct and concentrate techniques previously described.

OBSERVED PREVALENCES

E. histolytica was found in 118 of the 1,934 children examined in the survey, a prevalence of 6.1 per cent. This value was considerably below that which had been anticipated. Analysis of variance by race, sex, grade in school and location of the school (whether urban or rural) failed to show significant differences in prevalence. The details of this phase of the survey are to be reported subsequently.

In the positive family group of 208 persons examined in Winston-Salem, 43 individuals were found infected by *E. histolytica*, a prevalence of 20.7 per cent. Only one infected person was found in the control group of 160 individuals, a prevalence of 0.6 per cent. Infections by *E. histolytica*, therefore, were approximately thirty times more common among the family members of infected children than among the family members of children not found infected by this organism. Furthermore, these infections were over three times more frequent among the positive family members than among the entire group of 1,934

TABLE 1

Prevalence of Entamoeba histolytica in positive and negative control family members

Group	Number Examined	*Entamoeba histolytica*	
		Number	Per cent
Positive families			
Urban white	71	9	12.7
Urban Negro	137	34	24.8
Totals	208	43	20.7
Control families			
Urban white	53	0	0.0
Urban Negro	107	1	0.9
Totals	160	1	0.6

children examined in the survey of the schools. The findings in the positive and negative control families are shown in Table 1.

When the infected families are considered from the standpoint of race and geographical distribution within the City of Winston-Salem a significant difference appears. The prevalence of *E. histolytica* among the white and Negro children examined in the two schools utilized for this investigation of probands was practically identical, 6.4 per cent and 7.0 per cent, respectively. There were no demonstrable gross differences in the economic status of the two family groups under investigation and they may be considered comparable for the purposes of this study. The racial distribution within the two groups was not significantly different. Among the 208 persons examined in the positive family group, 137 (65.8 per cent) were Negroes; and of the 160 in the control group who were examined, 107 (66.9 per cent) were Negroes. Yet infections by *E. histolytica* were found to be almost twice as numerous in the families of infected Negro children as in the families of the infected white school children in Winston-Salem. Furthermore, the only infection in the negative control group was in an adult member of a Negro family.

Since other surveys have demonstrated that there is no statistically significant racial difference in the prevalence of infection by *E. histolytica* and since the overall findings of this survey are in agreement with these reports, some other explanation for this unusual racial prevalence of infection among Negro families must be found. There were two obvious possible explanations. The Negro families have lower standards of cleanliness and hygiene than the whites. This was not borne out by observations made of the homes by members of the survey team although crowding was frequently noted.

The alternate possibility was that the external sanitary environment of the Negro families was significantly lower than that of the whites and that it was accompanied by greater exposure to fecal contamination of food in the homes. The primitive housing conditions in certain of the areas concerned provided prima facie evidence of this possibility. The observed relationship between the prevalence of *E. histolytica* in the family groups and the domestic sanitary environment will be reported subsequently.

SUMMARY

Since age distribution analysis has shown that the prevalence of infection by *Entamoeba histolytica* reaches a maximum during school age and adolescence, the hypothesis that examinations of school children might yield evidence indicative of the prevalence of the infection in the community appeared justifiable. Such an investigation of a competent sample of the school population in Forsyth County, North Carolina revealed a prevalence of 6.1 per cent in the 1,934 school children examined. No significant differences in prevalence were observed with respect to racial distribution or residence. One group of infected urban children served as probands to the positive families and a second group of apparently uninfected urban children served as probands to the negative control families.

Examination of these two groups revealed a prevalence of *E. histolytica* of 20.7 per cent among members of the positive families and a prevalence of 0.6 per cent among the members of the negative control families.

These two comparable groups resided within the city limits of Winston-Salem, North Carolina. However, the observed prevalence of infection by *E. histolytica* was almost twice as great in the Negro families as in the white families. Since there is no evidence to indicate a special racial predilection to amebiasis among Negroes, this statistically significant difference may be attributable to greater exposure to infection and a poorer sanitary environment.

CONCLUSIONS

1. The observed prevalence of *Entamoeba histolytica* in a statistically valid sample of the school child population of Forsyth County, North Carolina was 6.1 per cent.

2. No significant differences in prevalence were observed with respect to race, sex or residence, whether urban or rural.

3. The observed prevalence of *E. histolytica* among the family members of urban children who were found to be infected was more than three times that of the observed prevalence among the total sample of 1,934 school children examined.

4. The observed prevalence of *E. histolytica* among the family members of infected urban children was more than 30 times greater than the observed prevalence in a statistically comparable group of family members of apparently uninfected urban school children.

5. These findings support the hypothesis that the examination of school children in Grades II through V provides a simple, relatively inexpensive and effective method of case-finding to determine the prevalence of amebiasis in a community.

ACKNOWLEDGMENT

The assistance of Mrs. Allen Holderman is gratefully acknowledged.

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FUMAGILLIN AND ERYTHROMYCIN IN THE TREATMENT OF AMEBIASIS[1]

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Two of the new antibiotics, fumagillin and erythromycin, have been found to possess amebicidal activity. Fumagillin kills *Entamoeba histolytica* in vitro in extremely low concentrations (McCowen *et al.*, 1951; Hrenoff and Nakamura, 1951) and has exhibited anti-amebic efficacy in man (Killough *et al.*, 1952; Anderson *et al.*, 1952). Anderson and his coworkers (*loc. cit.*) reported that fumagillin had no significant activity against the nonpathogenic protozoa infecting the human intestine, but Balamuth (1953) presented in vitro results which suggest a potent action against *Entamoeba coli*. Fumagillin has little effect on the intestinal flora, apparently acting directly on the amebae.

Erythromycin, with an antibacterial spectrum similar to penicillin's, has also evidenced anti-amebic action (McCowen *et al.*, 1953) which may be mediated through its effect on associated bacteria (Anderson *et al.*, 1954). Since action against amebae is thought to be indirect, erythromycin may be expected to provide results comparable to the tetracycline drugs, but results in a series studied by Anderson (*loc. cit.*) suggested that erythromycin may have greater action in vitro than indicated by in vitro studies with cultures of *E. histolytica*.

Preliminary clinical trials of fumagillin (Fumidil®) and erythromycin stearate (Erythrocin Stearate®) were carried out using natural infection in prisoners at the Mississippi State Penitentiary and patients at the Charity Hospital of Louisiana at New Orleans and the Tulane Tropical Medicine Clinic. Twenty-one patients, of whom fifteen had amebiasis, were treated with fumagillin (capsules, each containing 10 mg. of fumagillin) in a dose of 10 mg. three times daily for one week. Side effects occurred in nine patients but were not striking and did not warrant discontinuance of the drug. The following complaints from those taking the drug were recorded: nausea and abdominal pain three instances of each, diarrhea and headache twice each, abdominal discomfort and rectal pain once each.

After treatment stools were examined by direct smear and zinc sulfate centrifugal flotation. Three stools were examined within ten days after completion of treatment and additional specimens of fifteen patients were examined approximately six weeks later.

In general the treatment resulted in a temporary disappearance of amebae from the stools. One patient of the 21 still passed cysts of *Iodamoeba bütschlii* and another cysts of *Chilomastix* at the end of treatment. Trophozoites of *E. histolytica* never disappeared from the stools of one dysenteric patient nor did his

[1] This study was carried out with the assistance of funds and drugs granted by Abbott Laboratories.

TABLE 1

Results of treating intestinal protozoan infections in North Americans with fumagillin

Species	Number of Patients	Negative at End of Treatment	Relapsed
E. histolytica	15	14 (93%)	4 (27%)
E. coli	11	11 (100%)	2 (18%)
E. nana	8	8 (100%)	8 (100%)
I. bütschlii	3	2 (67%)	0
Chilomastix	1	0	0
Giardia	2	2 (100%)	0

symptoms subside with fumagillin therapy. Subsequent treatment with bismuth glycolylarsanilate provided a cure. At the end of six weeks *E. histolytica* had reappeared in the stools of 4 of 14 patients, *E. coli* in 2 of 11 and *E. nana* in 8 of 8. These findings are summarized in Table 1.

Eight adults were treated with 600 mg. of erythromycin every six hours for seven days. All developed diarrhea, which was not severe enough to require discontinuance of therapy or to interfere with work. Three of the patients were infected with *E. histolytica* alone; five with both *E. histolytica* and *E. coli*. All infections were eradicated and did not return during a follow-up period of six weeks.

Sixteen adults and children infected with *E. histolytica* were treated with 30 mg. of erythromycin per kilogram of body weight up to 1600 mg. per day in two to four doses for seven days. In two subjects both *E. histolytica* and *E. coli* were present; in the others *E. histolytica* alone. With the exception of one patient infected with *E. histolytica* neither *E. coli* nor *E. histolytica* could be found in the stools at the end of treatment, but in four cases *E. histolytica* reappeared in the stools in 14 to 40 days. There were no reported side effects in patients receiving this dose.

In four of these patients dysentery was the presenting complaint. In all four the symptoms were relieved within two days of initiation of treatment, but in one case amebae reappeared in the stools and dysentery recurred in two weeks.

In an effort to try fumagillin and erythromycin in a larger number of patients, a site was selected for study in Darien Province, Republic of Panama, where previous surveys indicated that the prevalence of amebiasis is considerably higher than in the United States (Faust, 1931a, 1931b). Headquarters were in the town of El Real on the Tuira River, and subjects were obtained from this and the nearby towns of Yaviza and Pinogana, as well as the surrounding portion of the Province.

The region is characterized by tropical rain forest broken by banana plantations and other farms. The population of El Real is about 1000, Yaviza 1500 and Pinogana 500. Medical care is dispensed at the District Dispensary, with a single physician for the entire district of about 400 square miles. Drinking water in El Real is obtained primarily by collection of rain and from the upper rapids of a small river, but additional water, undoubtedly polluted, is obtained from the river at its mouth where inhabitants also bathe and swim.

The segment of population studied was principally school children. Through the cooperation of the directresses and faculties of the public schools of the district all school children submitted one or more fecal specimens. Specimens from other individuals in the area from 14 months up to 54 years of age were examined at their request or when they were referred to the laboratory by the district physician.

Stools were processed as soon as possible after passage. Usually direct smear examinations were completed within one hour, but many of the specimens brought from Yaviza and Pinogana were as old as three hours before examination. In all instances both direct smears and a zinc sulfate centrifugal flotation technic were used (Beaver, 1952). Portions of each specimen were preserved routinely in formalin and PVA fixative (Brooke and Goldman, 1949) for later examination in New Orleans.

Individuals infected with *E. histolytica* were treated for one week with one of four sugar coated tablet preparations, each tablet containing respectively fumagillin 10 mg., erythromycin stearate 100 mg., fumagillin 10 mg. with erythromycin 100 mg., or fumagillin 5 mg. with erythromycin 150 mg. Dosage was as follows:

Fumagillin. Children through 5 years of age, 10 mg. per day; 6 through 10 years of age, 10 mg. twice a day; 11 years and older, 10 mg. three times a day.

Erythromycin. Children through 7 years of age, 100 mg. twice a day; 8 through 11 years of age, 200 mg. three times a day; 12 through 15 years of age, 200 mg. four times a day; adults, 300 mg. four times a day.

Fumagillin-erythromycin combinations. Dosage was governed by the content of fumagillin, which was given in the same amount as indicated above. Erythromycin was thus given in a ratio of 30 or 10 times the dose of fumagillin.

Stools were examined, if possible, three times within 10 days after completion of treatment, and again after an interval of 3 or 4 weeks. Specimens from 752 individuals were examined. All but 27 (3.5%) were found to have intestinal parasites. The incidence of parasitic infections is shown in Table 2.

One-hundred twenty Panamanian patients were treated for amebiasis with fumagillin, erythromycin or a combination of both drugs (Table 3). There was failure or recurrence of infection within seven weeks in over 15 per cent of 39 treated with fumagillin, no failure or recurrence in 18 treated with erythromycin and only one case of recurrence out of 63 patients treated concurrently with both drugs.

The most striking effect of treatment was the disappearance of both pathogenic and nonpathogenic amebae from the stools by the end of the seven-day course. *E. histolytica* was most readily eliminated, with *E. coli*, *Iodamoeba* and *E. nana* increasingly resistant in that order. The nonpathogenic amebae reappeared in the stools of 27 to 44 per cent of the patients during the succeeding six or seven weeks, and *E. histolytica* in about 6 per cent.

A proportionately greater number of failures (i.e., amebae in the stools at the termination of treatment) and recurrences (i.e., reappearance after a period of absence) were observed when fumagillin was used than with erythromycin alone

TABLE 2

Prevalence of intestinal parasites in 752 Panamanian patients

Parasite	Number Positive	Per cent Positive
Helminths		
Trichuris	538	71.5
Ascaris	524	69.6
Necator	352	46.8
Strongyloides	71	9.4
Enterobius	16	2.1
Protozoa		
E. coli	444	59
E. nana	374	49.7
I. bütschlii	249	33
E. histolytica	158	21
G. lamblia	148	19.7
C. mesnili	29	3.9
Trichomonas	21	2.8
Unidentified amebae	9	1.2
Balantidium	6	0.8
Dientamoeba	1	0.1
Other flagellates	56	7.4

or the combined treatment. The differences in results with fumagillin as compared with the other treatments in the case of each species of amebae are not statistically significant but it is nonetheless noteworthy that with each species of amebae fumagillin was the least successful of the four preparations.

In spite of the high incidence of amebiasis, amebic dysentery was encountered in only four persons. One of these was treated with fumagillin, and three with both fumagillin and erythromycin concurrently. In each case symptoms had abated within two days, the stools were negative by the end of the treatment period and amebae did not reappear during follow-up examinations.

Four patients with balantidiasis, all having diarrhea or dysentery, were treated. Two received fumagillin, another a course of fumagillin with erythromycin in a ratio of 1 to 10, and the fourth fumagillin followed by erythromycin. All treatments were unsuccessful.

Side effects of the drugs were not troublesome. Two of the patients receiving fumagillin alone complained of mild epigastric pain. There were no complaints from the individuals receiving erythromycin alone. Four persons receiving a combination of fumagillin and erythromycin complained of diarrhea, in one case severe enough to necessitate reducing the dose, and two complained of abdominal cramps.

The impracticality of long-term follow-up studies on unhospitalized patients in evaluating the efficacy of amebicidal drugs has been pointed out by Sodeman and Beaver (1952) and more recently by Killough and McGill (1954). Only one or two days may be required after inoculation for the passage of cysts of *E. histolytica* by an infected person (Walker and Sellards, 1913; Beaver *et al.*, 1954). Rendtorff (1954) has shown that at least in the case of the nonpathogenic amebae one cyst may suffice to initiate the infection. The value of the long-term follow-

up therefore varies inversely with opportunity for reinfection. This fact, illustrated by the data of Killough and McGill (*loc. cit.*), indicated the uselessness of multiple post-treatment stool examinations over a period of many weeks in the present study.

Affecting results in the opposite direction from the influence of reinfection is that of spontaneous cure, which in the case of *E. histolytica* has been shown to occur (Frick *et al.*, 1954; Beaver *et al.*, 1954) with significant frequency. Because of the small number of patients in the present series, no control group of untreated patients was set up, and rate of spontaneous loss of infection was therefore not determined.

Although based on a study of unhospitalized patients, the results obtained in the present study indicate that both fumagillin and erythromycin are useful amebicidal drugs. As employed, fumagillin was not as effective as previously reported (McHardy *et al.*, 1953), but the dosage used was only half of that prescribed by McHardy. More failures occurred with fumagillin treatment than with erythromycin or combinations of the two antibiotics. Although these differences were not subject to statistical evaluation due to the small number of cases, erythromycin, at least as the stearate compound, appears to be an amebicidal drug equal or even superior to fumagillin. Both drugs exhibit a definite effect on infections with the nonpathogenic amebae, which may or may not be temporary. This finding with regard to fumagillin corroborates Balamuth's (*loc. cit.*) in vitro results, in contradistinction to the broad spectrum tetracycline antibiotics which have little or no effect on the nonpathogenic amebae.

Efficacy of a drug against nonpathogenic amebae is of practical significance when the physician is forced to depend upon an unreliable laboratory for diagnosis. In such a situation many patients are subjected to course after course of antiamebic therapy directed against a nonpathogenic organism mistakenly identified as *E. histolytica*. Obviously it would be an immediate boon to the patient and at the same time release the physician's attention to other causes of the patient's symptoms to eradicate all of the intestinal protozoa, even if this were done with the mistaken idea that infection with *E. histolytica* were being treated.

In the preliminary and Panama studies a total of only nine cases of amebic dysentery was encountered. One of two receiving fumagillin, three of four receiving erythromycin, and three of three receiving a combination of both antibiotics were apparently cured, since symptoms were relieved and stools became negative for *E. histolytica*. In the others both symptoms and parasites persisted. These figures are too small to be significant, but correspond with the report by Killough and McGill (*loc. cit*) of increased resistance of the dysenteric infection to fumagillin. It appears that erythromycin may give rapid symptomatic relief in amebic dysentery. This finding is similar to that reported for carbomycin, chlortetracycline and oxytetracycline, and suggests that the proper place of these antibiotics in amebiasis may be as substitutes for the more toxic but highly effective emetine in the treatment of acute amebic dysentery. Emetine may still be necessary in an occasional patient (Sodeman *et al.*, 1952).

While both fumagillin and erythromycin appear to be useful amebicidal drugs, to be employed in situations where other drugs are contraindicated, i.e., failure

TABLE 3

Recurrence of amebae in stools of 120 Panamanian patients during various weeks after completion of treatment with fumagillin and erythromycin stearate

Species	Drug	Week							Total	%
		1	2	3	4	5	6	7		
E. histolytica	Fumagillin	2/23*	1/20	3/18	0/10	0/14	0/4	—	6/39	15.3
	Erythromycin	0/17	0/8	0/9	0/1	0/1	—	—	0/18	0
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{10}$	0/15	0/14	0/18	0/11	0/9	0/4	—	0/31	0
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{30}$	0/14	0/14	0/16	0/10	1/12	0/3	0/1	1/32	3.1
	Total	2/69	1/56	3/61	0/32	1/36	0/11	0/1	7/120	5.8
E. coli	Fumagillin	1/23	4/17	3/13	1/8	3/9	0/2		12/34	35.2
	Erythromycin	3/15	1/5	1/5	0/1	0/1	—		5/15	33.3
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{10}$	0/13	3/11	2/11	2/9	1/6	1/3		9/25	36
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{30}$	0/10	1/12	0/12	0/7	0/9	0/3		1/24	4.2
	Total	4/61	9/45	6/41	3/25	4/25	1/8		27/98	27.5
I. bütschlii	Fumagillin	3/8	1/7	3/10	2/5	2/7	0/1		11/19	57.8
	Erythromycin	4/13	0/3	0/4	0/1	0/1	—		4/13	30.7
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{10}$	0/8	1/5	2/12	1/8	0/5	0/2		4/19	21.0
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{30}$	0/5	0/5	0/5	0/3	0/7	—		0/11	0
	Total	7/34	2/20	5/31	3/17	2/20	0/3		19/62	30.6

E. nana	Fumagillin	5/17	5/14	3/11	3/5	3/6	0/1		19/30	63.3
	Erythromycin	1/12	0/5	0/5	0/1	1/1	—		2/12	16.7
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{10}$	0/10	0/6	2/13	1/5	1/3	0/1		4/16	25
	$\frac{\text{Fumagillin}}{\text{Erythromycin}} = \frac{1}{30}$	0/6	2/6	3/7	1/5	1/5	0/1		7/15	46.6
	Total	6/45	7/31	8/36	5/16	6/15	0/3		32/73	43.8

* Ratio of patients positive to patients examined.

or host sensitivity with other drugs, neither should be used indiscriminately. Fumagillin has been observed to produce leukopenia (Malewitz, 1953; Comer, 1955).

Erythromycin's efficacy in the treatment of penicillin-resistant staphylococcal infections is its most valuable feature and one that should be preserved if possible (Needham and Nichols, 1953; Dearing and Heilman, 1953). Routine use of this antibiotic in the treatment of amebiasis will increase the incidence of staphylococci resistant to both penicillin and erythromycin (Finland, 1954; Martin *et al.*, 1954). Since there are other effective drugs for routine treatment of amebiasis, erythromycin, in spite of the apparent amebicidal potency demonstrated in this study, should be reserved for use in cases when there are special indications.

SUMMARY

In preliminary trials of fumagillin and erythromycin in Louisiana and Mississippi, 45 patients were treated. Of fifteen patients with *E. histolytica* infection receiving fumagillin, four suffered recurrences. Eight persons with amebiasis treated with erythromycin 600 mg. every six hours were cured. Of sixteen individuals treated with 30 mg. per kilogram of body weight per day four suffered recurrences. Both drugs caused the temporary disappearance of nonpathogenic amebae from the stools.

One-hundred twenty Panamanian patients were treated for amebiasis with fumagillin, erythromycin or a combination of both drugs. There was failure or recurrence of infection within seven weeks in over 15 per cent of 39 treated with fumagillin, no failure or recurrence in 18 treated with erythromycin and only one recurrence out of 63 patients treated concurrently with both drugs. These differences were not statistically significant.

A definite effect upon nonpathogenic amebae was observed with each type of treatment but there was a high rate of recurrence. In the case of each species of amebae, *Endolimax nana*, *Entamoeba coli*, and *Iodamoeba bütschlii* there was a higher rate of recurrence with fumagillin treatment than with erythromycin or concurrent use of both drugs. These differences were not statistically significant.

Erythromycin provided rapid relief of amebic dysentery in three of four patients, fumagillin in one of two and combined therapy in three of three.

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Mrs. Cecilia Remon of the Republic of Panama and to Doctor Alberto Bissot, Director General of the Department of Public Health of that country for the opportunity to carry out a clinical study in Panama; to Doctor Julio Vega for his generous sharing of hospital facilities in El Real, and in particular to Mr. Pablo Othon for his indispensable help. Recognition is also made of the technical assistance of Miss Marjoree Jones and Mr. Franklin Sogandares and the cooperation of Doctor George Berryman of Abbott Laboratories, Inc.

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ERYTHROMYCIN IN AMEBIASIS[1]

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The amebicidal efficiency of certain antibiotic drugs has been established, but no single antibiotic agent has yet proved to be completely curative, nor have the therapeutic achievements even exceeded those attainable with nonantibiotic amebicides. With the introduction of each protozoicidal antibiotic drug, initial therapeutic acclamation has been enthusiastic. Time and further clinical experience have, in many instances, depreciated their status. The incidence of recurrences and objectionable side effects has prevented most antibiotic agents, when used alone, from being "ideal" amebicides.

Analysis of the extremes in therapeutic achievement reveals variance in susceptibility of parasitic strain and in severity of the disease, as well as inconsistency in mode of administration and inadequacy and lack of uniformity in follow-up examinations. Acquired alteration in bacterial and amebic sensitivity may also be hypothesized. Critical comparative review suggests that the antibiotic drugs have not impressively reduced the rate of recurrence below that reported for nonantibiotic amebicides. The trend today is toward use of combined antibiotic agents in therapy.

With nonantibiotic amebicides, the principal side effects are those idiosyncrasies related to the two metals, arsenic and bismuth, and the toxic effects of emetine on the cardiovascular system. Side effects associated with administration of antibiotic drugs are well recognized; gastrointestinal, dermal and mucosal reactions are frequently encountered when these drugs are administered in adequate dosage over a ten day period. Profound alteration in bacterial flora and development of resistant bacterial variants may occur with the wide spectrum agents. Hematologic influences are encountered less frequently. Protracted malaise is often an annoying and too casually discounted aftermath of antibiotic administration.

With such an unfavorable premise, justification for continued experimental and clinical evaluation of the newer antibiotic agents in terms of their protozoicidal influence may seem questionable. We rationalize our continued interest on the following bases:

(1) The ideal, complete amebicide is not yet available.
(2) The ideal combination of amebicides has not been established.
(3) The wide spectrum antibacterial antibiotic drugs offer a different therapeutic approach in amebiasis whereby host resistance and parasitic virulence may be influenced by alteration in bacterial flora, related to secondary infection, invasive aid, and nutritive supplement.

[1] Aided in part by a grant from Abbott Laboratories, North Chicago, Ill., and the Browne-McHardy Medical Group Research Fund, New Orleans, La.

(4) The direct and indirect actions of the antibiotic agents, especially those achieving well sustained concentrations of serum and essential tissue (liver), may, when coupled with the action of nonantibiotic amebicides, enhance curative incidence in both intestinal and extraintestinal amebiasis.
(5) The postamebic dysentery-irritable colon syndrome may be benefitted by control of the secondary bacterial infections.
(6) Symptomatic phases of amebiasis may be influenced by the bacterial flora (Ellenberg, 1946).

Erythromycin, isolated from *Streptomyces erythreus* and acclaimed as a protozoicide in preliminary experimental studies (McCowen *et al.*, 1953), has additional features that favor satisfactory clinical evaluation in amebiasis:

(1) On oral administration, it is rapidly absorbed and permits adequate seral concentrations for a period of six hours, thereby simplifying the mode of therapy.
(2) Since it is concentrated in the liver and is excreted biologically active in the bile, it may be effective in hepatic amebiasis.
(3) Its bacterial spectrum, similar to that of penicillin, is polyvalent but cannot properly be termed wide-spectrum. The gram-positive microbes are predominantly affected, and in this respect it is especially effective against strains of *Micrococcus pyogenes* (*Staphylococcus aureus*), which is resistant to the usual antibiotic drugs and is considered responsible for complicating enteritis associated with wide-spectrum antibiotic therapy (Dearing *et al.*, 1954; Nyhus*et al.*, 1954). The gram-negative intestinal flora, however, are not appreciably influenced.
(4) Its toxicity is recorded as minimal. Gastroenteric irritation, manifested by nausea, vomiting, abdominal pain and diarrhea, is, however, observed with excessive dosage.

The conclusion from clinical studies by McGuire and associates (1952) that "... of the essentially antibacterial antibiotics—only erythromycin appears to offer promise when used alone in drug-refractive amebiasis" was rather disconcerting, since they found erythromycin less effective than fumagillin in both *in vitro* and *in vivo* evaluation.

MATERIALS AND METHODS

All our studies were conducted on adults free of demonstrable extraintestinal involvement. No cases of acute amebic dysentery or any complications were included. Only 6 patients had proctosigmoidoscopically established amebic ulcerative changes; the remainder were cyst passers. Our initial clinical evaluation of erythromycin as an amebicide was abortive. The first 7 patients treated were refractory. They were later re-evaluated in terms of inadequate dosage and absorptive defect. All patients had been given 100 mg. of the drug every six hours for ten days. Some of the erythromycin base used was enteric-coated, and part of the supply was not coated for acid resistance.

In our next series of 8 patients we used erythromycin stearate (pediatric suspension), 200 mg. every six hours for ten days. This preparation had been

well endorsed (Nelson *et al.*, unpublished). Two patients showed persistence of *Entamoeba histolytica* at completion of treatment; a third demonstrated recurrence at the end of thirty days. The other 5 patients have remained free of parasitization through a six-month follow-up period.

Film-sealed erythromycin stearate tablets[2] then became available, and in all subsequent studies a schedule of 800 mg. of this preparation was employed daily for ten days. A total of 42 patients treated by this program has been followed in excess of six months. One had recurrence at thirty days, 3 at sixty days, and one at six months. No hematologic, renal or hepatic abnormalities were encountered on routine evaluation during therapy and ten days thereafter. Side effects, including nausea, abdominal cramping, mild diarrhea and pruritus ani, occurred in only 12 per cent of the patients and were limited to the therapeutic period. Mild post-therapeutic malaise persisted for an average of six days in all patients.

RESULTS

Our preliminary evaluation having been discounted as unsatisfactory, there remained 50 patients who received presumably adequate dosage of erythromycin stearate. Of these, 3 failed to exhibit clearance of *E. histolytica* at termination of treatment, and 6, all "cyst passers," showed recurrence after apparent clearance. We have made a reasonable effort to disprove reinfection. The therapeutic efficiency of erythromycin stearate in this study, 82 per cent, falls below the amebicidal efficiency estimated for two other antibiotic drugs, terramycin and fumagillin, and is appreciably lower than that of the commonly employed nonantibiotic amebicides. From our observations, erythromycin, when used alone, appears to be an innocuous amebicidal antibiotic agent whose therapeutic efficiency does not warrant its endorsement over other antibiotic and nonantibiotic amebicides that are more effective, even though more prone to side effects.

Lack of a consistently successful and complete amebicide, however, justifies evaluation of erythromycin administered concomitantly or in alternate courses with other amebicides. Several such previously reported investigations (McHardy *et al.*, 1954) have indicated that the efficiency of erythromycin is enhanced by combination with a nonantibiotic chemotherapeutic agent (Win 5047). Among 38 patients so treated and followed for an average period of sixty days, none demonstrated persistent parasitization at the end of therapy, and 3 showed recurrence at thirty days. The therapeutic efficiency, therefore, was 92.1 per cent.

DISCUSSION

Since no one available agent has been proved conclusively to be an infallibly successful amebicide, intensive search for a more effective drug is desirable. Evaluation of new drugs, however, is fraught with many difficulties. Dosage and duration of therapy can only be estimated. The numerous variables in parasite and host, the inability to differentiate between reinfection and recurrence, and

[2] Erythromycin stearate was supplied for use in this study by Abbott Laboratories, North Chicago, Illinois.

the occurrence of spontaneous remissions all make it difficult to establish the efficacy of any group, adequate control situations being so difficult to achieve.

In this study the estimated antiamebic efficacy of erythromycin was 82 per cent. On this basis it can be considered acceptable therapy only if more extensive studies in a larger series of patients or with altered therapeutic schedules yield a greater efficiency, or if its combination with a nonantibiotic amebicide enhances its therapeutic value. Relative freedom from side effects, absorbability, and wide tissue distribution favor its endorsement over combined nonantibiotic drugs. Failure to encounter a patient with untreated extraintestinal amebic involvement precluded our evaluation of the efficacy of erythromycin in such a case.

CONCLUSIONS

Erythromycin stearate is indicated in these studies to be antiamebic. More extensive clinical evaluation of erythromycin stearate administered alone and simultaneously with other antibiotic and nonantibiotic agents is indicated.

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SIMPLE ALIPHATIC SUBSTRATES IN THE CULTURE OF *ENTAMOEBA HISTOLYTICA*

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Strain 200 of *Entamoeba histolytica*, isolated by Tobie (1949), has been used in this laboratory to produce amebiasis in the guinea pig (Taylor, Greenberg, Highman, and Coatney, 1950). The infected guinea pigs have been useful in screening potential amebicides (Taylor and Greenberg, 1952). Limits are sometimes imposed on the usefulness of the method by seemingly random fluctuations in the infectivity of the amebae for the guinea pig. It was felt that perhaps if the Locke's-egg slant medium currently used for the cultivation of the amebae were replaced by a simpler, more defined and hence more reproducible medium, more uniform infections in the guinea pig would result. Efforts have previously been made to define the basal medium for the cultivation of amebae in the presence of other organisms (Hansen and Anderson, 1948; Hallman, Michaelson, and DeLamater, 1950), but these investigators, for the most part, used cultures containing a single bacterial associate. Luttermoser and Phillips (1952) have shown that strain 200 of *E. histolytica* loses its infectivity for the rabbit when cultured in the presence of a single microorganism. The same appears to be true in the guinea pig (unpublished observations of D. J. Taylor). For this reason it seemed preferable to retain for the present the mixed flora of strain 200 in any studies on factors affecting infectivity of the strain. The first problem was to devise as simple a medium as possible for the cultivation of this strain of amebae with a mixed bacterial flora.

A simple medium of egg albumin, rice powder, glucose, salts, and trace minerals failed in our hands to support growth of amebae beyond 48 hours even in the presence of a mixed bacterial flora. This medium became acid and it was felt that a carbonaceous substrate which did not yield acidic end products might favor growth of the organism. This report summarizes the results of culturing the amebae and bacteria with various sources of carbon in a medium containing egg albumin, salts, trace minerals, and rice powder.

MATERIALS AND METHODS

Strain 200 of *E. histolytica*, isolated originally by Tobie (1949), was used. This strain is routinely grown in Locke's-egg slant medium with rice starch added, and is transferred three to four times weekly. It is grown with a mixed and undetermined flora.

The basal medium used throughout these experiments is shown in Table 1. The proportions of salts are based on Locke's solution with added phosphate, decreased sodium chloride, and increased calcium chloride. We have no evidence that the amounts of any of the ingredients are optimum. The egg albumin was an impalpable powder, a commercial product of Merck and Company.

TABLE 1

Basal medium used for the culture of Entamoeba histolytica

Compound	Concentration
	mg./liter
NaCl	8,000
KH_2PO_4	450
$CaCl_2 \cdot 4H_2O$	250
$NaHCO_3$	200
$MnSO_4 \cdot 4H_2O$	1.25
$ZnSO_4 \cdot 7H_2O$	0.125
$CuSO_4 \cdot 5H_2O$	3.00
$Fe_2(SO_4)_3$ + Aq.	1.25
Egg albumin	2,500

The basal salt medium was made up in amounts of 1 to 4 liters and generally used within two weeks. Egg albumin was added immediately prior to preparation of the test medium. Test substances in the amount of 250 mg. were added to approximately 75 ml. of the albumin-salts medium, brought to pH 7.1 to 7.5 with hydrochloric acid or sodium hydroxide as required, and then made up to 100 ml.; the pH was redetermined at this point. Five milliliters of the final medium were added to 16 x 150 mm. cotton-stoppered test tubes, which were then autoclaved at 15 pounds pressure for 15 minutes. Sterile rice powder was added in the routine manner commonly used in the culture of amebae.

One to six substrates were tested in each experiment. As the results developed, series were arranged so that a previously successful substrate was retested along with several other substrates of undetermined value. One tube of each of the test media was inoculated with 0.5 ml. of the pooled and mixed bottom sediment from 24-hour cultures grown in the Locke's-egg slant rice starch medium. The cultures were incubated at 37° C. Maintenance of the culture was used as the criterion for the effectiveness of the medium. Harvests of bottom sediment were made every 48 hours and transferred to fresh tubes. A drop of the sediment was examined at each transfer and the presence or absence of living amebae noted. Cultures in which no amebae were found in samples taken from two successive transfers were discarded. In the early experiments, successful cultures were carried as long as the originally prepared medium lasted. It should be noted that no special effort was made at storing these tubes of media, which were kept at room temperature, unprotected from light, for as long as 40 days prior to inoculation, with no apparent appreciable deterioration. In later experiments, cultures were discontinued at the fifteenth transfer, since it was felt that further transfers would yield little information.

RESULTS

While there are some unexplainable variations in the data presented in Tables 1 and 2, certain patterns emerge. Fifty-two cultures were attempted using some 33 different substrates, and, in one trial, no substrate. Twenty cultures were maintained through 15 transfers over a period of 30 days. Sixteen different sub-

TABLE 2

Cultivation of Entamoeba histolytica in vitro *in a basal medium with various aliphatic substrates*

Substrate	Number Trials	Number Transfers with Living Amebae
None	1	0
Pyruvic acid	3	3, 1, 0
Citric acid	2	3, 2
Aconitic acid	2	0, 0
Succinic acid	2	0, 0
Fumaric acid	4	0, 15, 0, 1
l-Malic acid	3	5, 5, 4
Maleic acid	2	8, 8
Acetic acid	1	0
Acetamide	1	0
Mucic acid	1	0
d-Tartaric acid	1	0
d-Glucose	1	0
i-Inositol	1	1
n-Butanol	1	0
d-(+)-Glucosamine	3	15, 15, 15
Ethanolamine	3	15, 5, 15
i-Inosamine	2	15, 8
Choline	1	15
1-Amino-2-propanol	1	15
1-Amino-2-butanol	2	15, 15
2-Amino-1-butanol	1	15
4-Amino-1-butanol	1	15
4-Amino-1-pentanol	1	15
dl-Serine	1	0
dl-Threonine	1	0
Streptosamine	1	0
Methylamine	1	15
Dimethylamine	1	15
Ethylamine	2	1, 15
Diethylamine	1	15
Triethylamine	1	15
Di-*n*-propylamine	1	15
sec.-Butylamine	1	0

strates were used in these "successful" cultures, and all but one of these, fumaric acid, were aliphatic amines. The number of carbon atoms in these amines varied from 1 to 6; there were primary, secondary, and tertiary amines; and there were mono- and polyhydroxy amines, including glucosamine and inosamine.

There were 32 cultures which failed to survive 30 days. None of these was carried beyond the eighth transfer; 16 could not be maintained through one

transfer, and most of the rest survived no longer than the third transfer. Twenty substances were involved in unsuccessful cultures; 11 of these were carboxylic acids; 3 were alcohols (glucose, inositol, and *n*-butanol); 2 were hydroxyamino acids (serine and threonine); and 7, including the last two, could be classified as amines. Of the 7 amines which failed to maintain the amebae, 4 were tested once (serine, threonine, *sec.*-butylamine, and streptosamine), and 3 were found in other tests to be capable of supporting the growth of amebae through 15 transfers.

While there seemed to be some specificity in the requirement for aliphatic amines, within the group of amines tested there appeared to be no rigid requirements. Cultures with amines were not always successful, but in about 85 per cent of the trials the amebae could be maintained for at least a month. In contrast, only one culture of the 25 employing non-amino compounds was successful by our definition. This was a culture in which fumaric acid was the substrate. No reasonable explanation can be offered for this one exception, especially in view of the fact that in three retrials of fumaric acid the amebae did not survive beyond the first transfer.

The following are offered as possible explanations for the success achieved with amines: (1) they serve as a buffer as well as a source of carbon and nitrogen, (2) they yield a common metabolite required by the amebae, or (3) they preferentially support the growth of a limited number of organisms best suited for the growth of amebae.

Again it should be pointed out that it is not known whether the concentrations of substances used in the media were optimal.

SUMMARY

Entamoeba histolytica with its accompanying bacterial flora was cultured in vitro in a basal medium of inorganic salts and egg albumin plus rice starch, to which was added a variety of aliphatic amines and amino alcohols. In general, other simple substrates, including glucose and other alcohols, carboxylic acids, and certain amino acids failed to support the growth of the amebae.

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OBSERVATION OF PYRIMETHAMINE (DARAPRIM) AS A SUPPRESSANT OF MALARIA IN A SMALL VILLAGE IN JORDAN*

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Vincke (1952) was one of the first to test and report the value of pyrimethamine (2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine) as a suppressant of malaria. The study was carried out in 10 isolated villages in the Belgian Congo. Each villager received a single 25 mg. dose weekly for 25 weeks. When the experiment was started 22 per cent of the population harbored malaria parasites, but at the end of the fifteenth week no parasites could be found. The suppressive action of this drug has been demonstrated also by Goodwin (1952) and by Coatney *et al.* (1953).

The study here reported was carried out to secure further information on the efficacy of pyrimethamine as a suppressant of malaria under field conditions and to demonstrate the value of the drug in the locality of Safut, a small village 10 miles north of Amman, where malaria was known to be endemic. Originally plans were to extend the investigation to another village a few miles away, but they failed to materialize because of lack of full cooperation on the part of villagers. Observations covered a period of more than four months (June 20 to October 24) and coincided approximately with the malaria season in the area. The time also coincided with the harvest period, when most villagers slept outdoors in open tents.

DESCRIPTION OF THE LOCALITY

Safut is a village of about 300 inhabitants, mostly Arab Christians. It is located at an elevation of about 2,800 feet. The mean rainfall is approximately 13 inches a year. There is a small stream about one-half mile east of the village, and several other small fresh water springs open in and nearby the village. Mosquito larvae collected in these waters were identified as *Anopheles sergenti*, *A. superpictus*, *A. claviger* and *A. marteri*. Adult mosquitoes collected from stables and houses in the village were determined as *A. superpictus*. The incidence of malaria in the village was not definitely known, but the villagers complained of many cases during the warmer months of the year.

Safut is one of the many hill villages where malaria is endemic. The incidence of malaria in these villages varies from year to year, a condition undoubtedly dependent upon the amount of available surface water. As discussed by Leeson

* Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 339.

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et al. (1950), malaria is more prevalent after late and heavy winter rainfall which provides a sustained supply of spring water available for mosquito breeding. In the Jordan Valley, north of the Dead Sea, and in the Wadi 'Araba, south of the Dead Sea, malaria is hyperendemic (Leeson *et al.*, 1950). In these areas the incidence of malaria probably varies little from year to year owing to the abundance of surface water and warm temperature which insure continuous mosquito breeding.

METHODS AND MATERIALS

In carrying out this investigation, the survey team usually consisted of three people. The first person recorded the necessary data on each villager, the second made blood smears, and the third administered the drug.

At the beginning of the study two duplicate cards were prepared for each villager. On each card was recorded the number, name, sex, age and treatment, (i.e., Daraprim or a placebo). The group of people that received placebos served as the control. No selection was made as to who was to receive placebos. The duplicate registration cards were prepared in advance in such way that the word "Daraprim" or "Placebo" occurred alternately; in this way about half of the people received the drug and the other half placebos. After registration cards were prepared, one card was given to each villager to bring on each subsequent visit, and the duplicate card was kept by us. In addition, a master card was kept for each individual on which all the above information was recorded plus clinical history and the result of the blood examination.

All the villagers who reported at the first meeting had thick and thin smears made of their blood; subsequent smears were taken every six weeks. The smears were prepared and stained with Giemsa after the method of Barber and Komp (1924). Most of these smears were examined by the authors. Usually 100 or more fields were examined before a slide was considered negative.

The administration of the drug or the placebo was begun on the day of registration, and repeated on the same day once every week. Children up to 14 years of age received one-half (12.5 mg.) a tablet, and those 15 years or older received one tablet (25 mg.). Small children were given crushed tablets in water. Every effort was made to make sure that the drug was actually swallowed. Individuals who developed malaria during the course of the study were treated with chloroquine. Adults received 900 mg. in two doses for the first day, and 300 mg. on each of two subsequent days. Children received one-half of the adult dosage.

OBSERVATIONS AND RESULTS

Two hundred and fifty individuals were registered at the beginning of the study in June. Malaria parasites were found in 28, or 12 per cent. All the parasites were identified as *Plasmodium vivax*. Of the above number of persons, 90 were children between the ages of 2 and 9. The parasite rate among the children was 16.2 per cent. During the course of the 18-week study, 20 of the villagers moved away. Therefore, final results of the study are based on the remaining 230 people.

Among those who remained throughout the study, were nine children less than 2 years old, 83 from 2 to 9 years of age, 37 from 10 to 14 years of age, and 101 who were 15 years or older. One hundred and ten persons (test group) received a weekly dose of pyrimethamine, and 120 (control group) received placebos. Among the test group, 14 or 12.7 per cent were positive for malaria before treatment started. In the control group, 14 or 11.6 per cent were positive, also. In the first study made six weeks following the start of treatment, the incidence of malaria among the test group was reduced to zero. No malaria parasites were found in the blood films of the test group made every six weeks thereafter until the end of the study. This indicated that the drug effectively suppressed malaria at the dosage used.

During the 18 weeks of observation, 25 persons in the control group who were negative for malaria at the beginning of the study developed malaria (*P. vivax*). To what extent these represented relapses or were new cases of malaria, we were not able to determine. All 14 individuals of the control group who were positive for malaria at the beginning of the study were found negative by the 18th week when the study was terminated.

SUMMARY

Pyrimethamine (Daraprim) in doses of 25 mg. per adult, was administered weekly to 110 individuals living in a small malarial endemic village in Jordan. One hundred and twenty other individuals living in the same village who served as the control group received placebos. During the 18-week period of the study (June 20 to October 24, 1954), 25 of the control group developed malaria (*P. vivax*) whereas no cases of malaria occurred among the pyrimethamine treated individuals.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Mr. John Hadjinicolau, UNRWA Entomologist, for collecting and identifying the mosquitoes in and around Safut, and to Mr. Edmond Sarkissian for assistance in the examination of some of the blood smears.

We wish to express our thanks also to Dr. G. R. Coatney of the National Institutes of Health for assistance and for advice in preparing this manuscript, and to Burroughs Wellcome and Company for supplying tablets of Daraprim and placebos used in this study.

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TRYPANOSOMA CRUZI INFECTION IN THE INDIAN MONKEY[1]

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A recent report of trypanosomiasis in the monkey (Wolf *et al.*, 1953) has reviewed the recorded instances of simian infection by such flagellates. It describes the activation of a latent trypanosomiasis in a number of rhesus monkeys of Indian origin following the prolonged administration of cortisone. The substance was given in a successful attempt to abort the development of experimental disseminated encephalomyelitis produced by the injection of emulsions of homologous brain and the Freund adjuvants (Kabat *et al.*, 1952). Motile flagellates found in the blood of a few monkeys averaged 19–20 micra in length and had a large, posterior kinetoplast, very fine, granular, lightly basophilic cytoplasm, a good-sized flagellum and a narrow, undulating membrane. In the group of actively infected monkeys, lesions predominated in the central nervous system and heart. Leishmaniform organisms were present in the epithelioid cells of the numerous granulomas in the neural tissue, and in the muscle fibers of the zones of interstitial myocarditis, as well as in the less involved skeletal muscle, bone marrow and fat. Lesions were rarely encountered in the intestinal wall, liver and lymph nodes. Because of the appearance of the flagellate, the occurrence of a leishmaniform stage and the morphology and localization of the lesions, the micro-organism was tentatively identified as *Trypanosoma cruzi* although no definite conclusion was drawn. Unfortunately, by the time the pathological tissues were seen microscopically no suitable material for transmission of the infection was available. The following procedures were then carried out to isolate and identify this micro-organism.

MATERIAL AND METHODS

Since it had been noted that clusters of leishmaniform organisms (as well as clusters of sarcosporidia) were especially abundant in muscle fibers in and near the inoculation sites, having apparently been stimulated to local multiplication, muscle tissue adjacent to an inoculation site in the lateral portion of the right thigh was removed aseptically from a rhesus monkey of Indian origin (S-98-8) when it was sacrificed with ether. This monkey had received six intramuscular inoculations of an emulsion of adult monkey brain with adjuvant material over a period of 4 months. This animal did not develop experimental allergic encephalomyelitis. Approximately three grams of fresh, grossly normal, striated muscle tissue was cut into fine strips with scissors and ground lightly in a mortar. This material was suspended in five mls. of sterile physiological saline solution.

Cultivation. The monkey muscle suspension was inoculated into Seneca hemoflagellate medium which was prepared as follows: To 50 grams of Bacto beef

[1] Aided by a grant from the Matheson Commission.

(Difco) 1000 mls. of distilled water was added and heated at 50°C. for one hour, and then at 80°C for five minutes. It was then filtered through filter paper and the following ingredients added: Neopeptone—20 gms; Nobel agar—20 gms; NaCl C. P.—5 gms. The pH was adjusted to 7.2 to 7.4 and the medium was then autoclaved at 15 lbs. for 15 minutes, cooled to 45°C., and defibrinated rabbit blood was added to make 10% of the whole. It was then pipetted in 3 ml. amounts into sterile test tubes and solidified in a slanting position on ice. An overlay of 2 ml. of sterile solution was added. Twenty thousand units of penicillin were added to each tube inoculated with the suspension of monkey tissue to control the growth of bacteria. The tubes were incubated at room temperature (22–28°) and their contents were examined at the end of one and two weeks of incubation. Subcultures were made at intervals of 10 to 15 days.

Transmission of the infection to mice. Four-to-six-weeks-old dba mice were used routinely in these studies, since Swiss white mice were known to be resistant to infection with *Trypanosoma cruzi*. The mice were injected intraperitoneally with one-to-two-week-old cultures of trypanosomes obtained by the methods used above. The blood was examined daily for trypanosomes.

Transmission of infection to triatomid bugs. Laboratory raised, infection free *Triatoma gerstaekeri* and *Rhodnius prolixus* were kindly supplied by Dr. A. Pachchanian of the University of Texas, Galveston, Texas. Twenty *R. prolixus* and twelve *T. gerstaekeri* were fed on an infected dba mouse. The feces of the triatomids were examined at frequent intervals for the presence of trypanosomes and crithidia. During the course of these tests some of the triatomids died.

Serological studies. Preparation of the antigen. Two standard *Trypanosoma cruzi* strains (1114 and Lilly) and the Indian strain were grown on Seneca hemoflagellate medium. They were then grown for ten to twelve days on the N I H modification of the Boeck-Drbohlav's medium containing 2.5 ml. of buffered saline solution overlay. The culture was harvested, and 0.1 ml. of 10% formalin was added to 50 ml. of pooled culture. The antigen was standardized to contain about 7.5 million trypanosomes per ml. by counting the trypanosomes in a hemocytometer.

Immunization of rabbits. Two adults rabbits were immunized against *T. cruzi* 1114; another two against the Lilly strain and two others against the Indian strain. The first dose was 0.25 ml. and the subsequent ones 0.5 ml. of the antigen for a total of fourteen doses administered twice a week. Half a ml. of live trypanosome cultures were injected once a week for 7 weeks. All injections were given subcutaneously or intramuscularly. One hundred and ten days after the initial injection, the rabbits were bled aseptically from the heart. The serum was separated under sterile conditions and kept in the ice box for agglutination and agglutination absorption tests.

Agglutination tests. The serum from each rabbit was serially diluted beginning with 1:10 and ending with a 1:5120 dilution and tested for agglutinins using the above mentioned three antigens. The tubes were then incubated overnight at 37°C. and readings were taken.

Agglutination absorption tests. To a 1:5 dilution of *Trypanosoma cruzi* (1114) immune serum, an equal volume of *T. cruzi* (1114) antigen was added so that

the final dilution of serum was 1:10. Similarly, to *T. cruzi* (1114) immune serum, an equal volume of T. cruzi (Lilly) antigen was added. In a like manner an equal volume of Indian trypanosome antigen was added to *T. cruzi* (1114) immune serum. One thousand units of penicillin were added to each tube to prevent bacterial contamination. The tubes were then incubated overnight, and kept for another day in the ice box. To a 1:5 dilution of *T. cruzi* (Lilly) immune serum, an equal volume of Indian trypanosome antigen, *T. cruzi* (1114) or *T. cruzi* (Lilly) antigen were added. Similarly, to a 1:5 dilution of Indian trypanosome immune serum an equal volume of Indian trypanosome antigen or *T. cruzi* (1114) antigen or *T. cruzi* (Lilly) antigen was added. After adding penicillin as above, the tubes were incubated overnight at 37°C., and then kept another day in the ice box. The immune sera plus antigen were then centrifuged and the supernatant fluid separated under sterile conditions. Each serum (1:10) which had been absorbed by the homologous and heterologous antigen was then tested against the homologous and heterologous antigen by the usual tube dilution method of agglutination in 1:20, 1:40, 1:80 and 1:160 dilutions. The racks were incubated at 37°C., overnight, and readings were then taken.

RESULTS

Cultivation. On the Seneca flagellate medium, the Indian trypanosome grew within a week to ten days. Smears showed the presence of both crithidia and trypanosome forms. They also grew very well on Boeck-Drbohlav's egg medium. At first the growth was poor, but in the subcultures it became very rich. At present the subcultures are maintained by transferring them twice a month. In older cultures the trypanosomes have a tendency to become rounded and sluggish. In the egg medium the growth in the saline overlay was very homogeneous, and there was no tendency to clumping in the young cultures.

Infection of mice. The four-to-six-week-old dba mice inoculated intraperitoneally with one-to-two-week-old cultures developed a mild parasitemia in seven to ten days. All the mice recovered. In the peripheral blood no more than 2 trypanosomes were seen per high power field. When 2.5 mg. of cortisone was injected subcutaneously, and repeated in 24 hours, a fatal trypanosomiasis resulted. The parasite appeared in the blood in 3–5 days, their number gradually increased to 20–30 trypanosomes per high power field, and all mice died in 2–3 weeks. In wet preparations the trypanosomes were hypermotile, manifesting a progressive forward and undulating movement. In stained smears, they had the characteristic C-shaped forms, and measured about 20 micra in length. The cytoplasm stained blue, and the kinetoplast and flagellum red or violet with Wright's stain. There were long, slender forms, as well as short and broad forms, the nucleus was centrally located and the kinetoplast was situated at the posterior end.

Transmission of the infection to triatomid bugs. On April 23, 1954, twenty *Rhodnius prolixus* and twelve *Triatoma gerstaekeri* were fed on an infected dba mouse. On May 10, all living bugs were examined for the presence of trypanosomes in their rectal discharges. Only one *R. prolixus* had trypanosomes and crithidia in its feces (17 days after the initial feeding). None of the *T. gerstaekeri*

were found to be infected. The bugs were fed on a normal guinea pig about once every ten days. On May 18 (25 days after the initial feeding) two more *R. prolixus* were found to be infected. On May 25, there were only three living *T. gerstaekeri*, and one was found to be infected (32 days after the initial feeding). There were numerous trypanosomes and crithidia in the feces. Many of the bugs died following vigorous manipulation in an attempt to obtain fecal material. The migration of the Indian trypanosome in the triatomid bugs was caudal or rectal with none to the cephalic or pharyngeal region.

Serological findings, agglutination tests. Table 1 shows the results of agglutination tests of *T. cruzi* (Lilly), *T. cruzi* (1114) and Indian trypanosome rabbit

TABLE 1

Cross agglutination tests of three rabbit immune sera against the same three trypanosome strains

Antigen	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
T. cruzi (Lilly) antiserum—Rabbit 1										
T. cruzi (1114)	+	+	+	+	+	+	+	−	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	−	−	−
Indian trypanosome.	+	+	+	+	+	+	−	−	−	−
T. cruzi (Lilly) antiserum—Rabbit 2										
T. cruzi (1114).....	+	+	+	+	+	+	+	−	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	−	−	−
Indian trypanosome..	+	+	+	+	+	+	+	−	−	−
T. cruzi (1114) antiserum—Rabbit 1										
T. cruzi (1114).....	+	+	+	+	+	+	+	+	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	+	−	−
Indian trypanosome..	+	+	+	+	+	+	+	−	−	−
T. cruzi (1114) antiserum—Rabbit 2										
T. cruzi (1114).....	+	+	+	+	+	+	+	+	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	−	−	−
Indian trypanosome..	+	+	+	+	+	+	+	+	−	−
Indian trypanosome antiserum—Rabbit 1										
T. cruzi (1114).....	+	+	+	+	+	+	+	+	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	+	−	−
Indian trypanosome..	+	+	+	+	+	+	+	+	−	−
Indian trypanosome antiserum—Rabbit 2										
T. cruzi (1114).....	+	+	+	+	+	+	+	+	−	−
T. cruzi (Lilly).....	+	+	+	+	+	+	+	+	−	−
Indian trypanosome..	+	+	+	+	+	+	+	+	−	−

+ means agglutination; − means no agglutination.

immune serum against *T. cruzi* (Lilly), *T. cruzi* (1114) and Indian trypanosome antigens. *T. cruzi* (Lilly) immune serum from rabbit No. 1 agglutinated *T. cruzi* (1114) and *T. cruzi* (Lilly) in 1:640 dilution, and the Indian trypanosome in 1:320 dilution. *T. cruzi* (Lilly) immune serum from rabbit No. 2 agglutinated the three trypanosomes in 1:640 dilution. *T. cruzi* (1114) immune serum from rabbit No. 1 agglutinated 1114 and Indian trypanosomes in 1:1280 dilution, and the Indian trypanosome in 1:640 dilution, while immune serum from rabbit No. 2 agglutinated the 1114 and Indian trypanosomes in 1:1280 and the Lilly trypanosome in 1:640 dilution. Immune serum from rabbits No. 1 and No. 2 prepared against the Indian trypanosome agglutinated the Indian, Lilly and 1114 trypanosomes in 1:1280 dilution.

Agglutination absorption tests. The trypanosomes of the known *T. cruzi* strains (1114 and Lilly) completely absorbed their own agglutinins as well as the agglutinin of the Indian trypanosome. Similarly, the Indian trypanosome completely absorbed its own agglutinin as well as the agglutinins of the two known *T. cruzi* stains (1114 and Lilly).

COMMENT

Trypanosoma cruzi has been thought by some to be present only in the Americas. The present data support the view that it may be found in the Eastern hemisphere. One of the two original descriptions of spontaneous simian trypanosomiasis was that of Brumpt (1909) who described an infection in a young cynomolgus monkey from Java. Although he at first named it *Trypanosoma vickersae*, he later (1949) stated that he believed it to be *Trypanosoma cruzi*. Terry (1911) described a trypanosome, which he named *Trypanosoma rhesi*, in a group of Indian rhesus monkeys; Brumpt (1949) subsequently classed this micro-organism with his own as *Trypanosoma cruzi*. Malamos (1935) encountered similar flagellates in Javanese cynomolgus monkeys and transmitted the infection to monkeys, mice and rats; he found that *Triatoma infestans* could be infected with this micro-organism by blood sucking. He concluded that the flagellate was *Schizotrypanum cruzi* and Brumpt (1949) later included it with his own and Terry's examples as an instance of *Trypanosoma cruzi*. Fulton and Harrison (1946) found trypanosomes in the blood of rhesus monkeys of Indian origin and reported that they were indistinguishable from a strain of *Trypanosoma cruzi* maintained in their laboratory.

SUMMARY

A flagellate encountered incidentally in Indian monkeys in the course of an investigation of experimental disseminated encephalomyelitis (Kabat *et al.* 1952) had been identified tentatively as *Trypanosoma cruzi* (Wolf *et al.* 1953). This was based upon the appearance of the micro-organism as a trypanosome having the morphological features of *T. cruzi* in the peripheral blood, its occurrence in the tissue in a leishmaniform stage, and upon the nature and distribution of the lesions produced by the flagellate in the monkey.

The present studies have shown that the trypanosome cultured from the

skeletal muscle of a rhesus monkey imported from India is identical with *T. cruzi* of the Americas. This conclusion is based upon the following data: 1) the cultural characteristics of the Indian trypanosome are identical with those of *Trypanosoma cruzi*; 2) biologically, it passes through the leishmaniform, leptomonad, crithidia and trypanosome forms in the vertebrate and invertebrate host and in cultures; 3) serologically, it has the same agglutinins as two standard American *T. cruzi* strains (1114 and Lilly) and there is complete cross-absorption of agglutinins between it and these two strains of *T. cruzi*; 4) two species of triatomid bugs, *Triatoma gerstaekeri* and *Rhodnius prolixus* have been successfully infected with the Indian trypanosome; trypanosomes first appeared in the rectal discharges of the bugs 32 and 17 days respectively after the infective feeding.

CONCLUSION

A trypanosome observed in rhesus monkeys imported from India is *Trypanosoma cruzi* of Chagas.

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STUDIES ON THE DYNAMICS OF EXPERIMENTAL TRANSMISSION OF WEST NILE VIRUS BY *CULEX MOLESTUS*

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West Nile infection is widespread in the Middle East, giving rise to inapparent or unrecognized infections in Egypt (Melnick *et al.*, 1951; Taylor, 1953), and to an epidemic disease, West Nile fever, in Israel (Bernkopf *et al.*, 1953; Goldblum *et al.*, 1954). Taylor and Hurlbut (1953) have suggested that in nature the infection is transmitted to man by culicine mosquitoes. They recovered West Nile virus in seven pools of *Culex antennatus*, *Culex pipiens* and *Culex univittatus* out of 300 pools of these species of mosquitoes tested. These authors also demonstrated that *C. antennatus* and *C. pipiens* become infected when fed on infant mice circulating West Nile virus and transmit the infection after a suitable incubation period to normal infant mice. Circumstantial evidence in favor of mosquitoes, and particularly *Culex molestus*, being involved in the transmission of West Nile virus have also been obtained in this country. Olejnik (Eylan), 1952, isolated a virus from *Culex molestus* caught in nature and succeeded in transmitting it from experimentally infected to healthy hamsters by the bite of laboratory bred *Culex molestus*. This virus has later been identified as West Nile virus (Eylan, 1954). Successful experimental transmission of West Nile virus by aëdine as well as culicine mosquitoes has also been reported from other laboratories (Philip and Smadel, 1943; Kitaoka, 1950; Goldwasser and Davies, 1953; Davies and Yoshpe-Purer, 1954).

Since *Culex molestus* is the most widespread house-dwelling culicine in Israel, and is abundant in areas where West Nile fever occurs, it was deemed desirable to investigate more thoroughly the mechanism of infection of *Culex molestus* with West Nile virus and the transmission of this virus to susceptible animals by the bite of experimentally infected mosquitoes. The present study extends the observations reported by other workers and deals with certain factors of general importance in the transmission of a virus by mosquitoes.

MATERIALS AND METHODS

Virus. Strains of West Nile virus recently isolated from the blood of West Nile fever patients were used throughout these experiments. These strains have been through few mouse brain-to-brain passages. Usually the 5th or 6th mouse brain passage virus was used. Earlier mouse brain passages were not used since constant high titers were obtained only at the 3rd to 4th mouse passage or even later. Virus was employed as a brain suspension in saline containing 10 per cent normal rabbit serum. The stock suspensions of 10 per cent infected mouse brains

[1] The authors are indebted to Dr. Joseph L. Melnick, Yale University School of Medicine, New Haven, for helpful advice and criticism. The technical assistance of Miss Miriam Polak, Miss Erna Klein, Miss Pnina Shpigler, and Mr. Joel Medina is gratefully acknowledged.

yielded titers between 10^{-7} and 10^{-8}. The concentrations for feeding mosquitoes were obtained by diluting the suspensions to required titer. The virus suspension was added to defibrinated normal rabbit blood in proportion of 1 to 3 by volume. Small Petri dishes each containing approximately 7 ml. of this mixture soaked on cotton were introduced into the mosquito cages having dimensions of 50 x 50 x 80 cm. each. In order to obtain accurate titers of the suspensions, the blood virus mixtures immediately after their feeding to mosquitoes were diluted in tenfold dilutions and inoculated intracerebrally into 3–4 week old Swiss mice. The fifty per cent endpoint titer was determined by the method of Reed and Muench (1938).

Mosquitoes. The mosquitoes used were laboratory reared *Culex molestus.* The stock originated from a number of females collected in the field during the summer of 1953. The larvae were reared in shallow dishes on bread infusion. Adults were provided with water and 50% sugar solution. Both larvae and adult mosquitoes were kept at a temperature of 31°C (±1°C.) and 50% (±5%) relative humidity until used. *Culex molestus* has the ability to propagate autogenically and is not a ready biter in captivity under laboratory conditions. However, by selection of the progeny from females fed on laboratory animals, a strain was obtained which could be induced to bite quite willingly. This mosquito strain was used throughout.

Feeding of mosquitoes on suspensions of virus. The usual procedure of an experiment was as follows: three- to seven-day old mosquitoes were used. Water and sugar were withdrawn 24 hours before the beginning of the experiment. Petri dishes containing the blood-virus mixture were distributed in the cages in such a way that 7 ml. of mixture were offered to approximately 500 mosquitoes. On the average 50–60 per cent of the females fed within one hour, the time limit imposed in order to minimize deterioration of virus. Following the infectious meal, all mosquitoes were immobilized by carbon dioxide anesthesia, the engorged females separated and transferred in groups of 100 to holding cages which were kept at a temperature of 28°C. (±1°C.) and a relative humidity of 65% (±5%).

To determine the virus content of the mosquitoes, samples of about 50 mosquitoes, before or after having fed on infant mice, were sacrificed at varying intervals of time following the infectious meal. The mosquitoes were triturated in saline containing 10% normal rabbit serum with the addition of 10 mg. and 500 units per ml. of streptomycin and penicillin respectively, and the suspensions thus obtained were titrated intracerebrally in 3–4 week old mice. The preparation of these suspensions was based on mosquito weight which had been determined as being on the average 2 mg. for an unfed and 2.8 mg. for an engorged mosquito.

Transmission of virus to mice by infected mosquitoes. In order to determine the ability of the mosquitoes to transfer the virus to susceptible animals by bite, infant mice 1–3 days old were introduced, at varying intervals of time following the infectious meal, into the cages holding the infected mosquitoes. Infant mice were chosen for this purpose, because of their high susceptibility to extraneural

routes of infection with West Nile virus. The mice were gently tied to a board so that they were unable to repel the mosquitoes. After three hours of exposure to random bites of infected mosquitoes, the mice were returned to their mothers and kept under observation for a period of two weeks. On the average 3–6 mosquitoes fed on an infant mouse. Infant mice showing signs of CNS involvement after being bitten were sacrificed, their brains harvested and passaged intracerebrally to adult mice. After two additional intracerebral passages in adult mice the virus was identified by challenge of hamsters immunized against West Nile virus.

In certain of the experiments infant mice were bitten by single mosquitoes. This was accomplished in the following way: when one mosquito settled to feed on an infant mouse, the mouse was enclosed in a wide-mouth glass tube, covered with gauze on the upper side. Thus, other insects were prevented from approaching the mouse and the intake of blood by the mosquito could be easily observed.

EXPERIMENTAL RESULTS

Infection of Culex molestus *and transmission of infection to infant mice.* Table 1 presents results of West Nile infection of mosquitoes and their transmission of virus to infant mice on different days after feeding on mouse brain suspensions of varying titers. Three experiments were carried out at different times and the data are averaged in the table. Suspensions for feeding were prepared using 100-fold differences in virus concentration. Infected mouse brain suspensions were employed at concentrations of 10^{-1}, 10^{-3}, and 10^{-5}. Infectivity titrations of the actual suspensions fed were run in serial 10-fold dilutions only after the mosquitoes had fed for one hour. It will be seen that with suspensions having titers of $10^{-6.6}$ and $10^{-4.3}$, virus was transmitted by mosquitoes to infant mice after an extrinsic incubation period of 7–21 days, whereas when the titer of the suspension fed was $10^{-2.8}$, no transmission occurred. The results suggest a graded relation between the length of the extrinsic incubation period and the percentage of mice infected by the bite of mosquitoes. Both, at $10^{-6.6}$ and $10^{-4.3}$, one week following the infectious meal the number of mice infected was low but the percentage of mice infected increased with the longer incubation periods of 2 to 3 weeks.

It should be mentioned that the percentage of mosquitoes feeding on mice was the lowest on the 7th day after the infectious meal. This may be attributed to the fact that a large number of mosquitoes were still gravid on that day or had just laid their eggs, and therefore, were unwilling to bite. From the 9th to the 21st day, the percentage of mosquitoes feeding on mice fluctuated in different experiments between 20–40 per cent. After 21 days, natural mortality was so high that few mosquitoes remained alive.

At different time intervals following the infectious meal, groups of 50 mosquitoes were sacrificed and their virus titer determined. Preliminary experiments showed only slight differences, amounting to less than one log in titer between engorged and nonengorged mosquitoes of the same group. The last column in Table 1 gives titers of virus in mosquitoes at the different extrinsic incubation

TABLE 1

Transmission of West Nile virus by Culex molestus *infected by feeding on virus suspensions of varying infectivity*

Titer of Suspension Fed to Mosquitoes	Days Following Infectious Meal	No. of Infected Mosquitoes to Which Infant Mice were Exposed	No. of Mosquitoes Which Fed on Mice	No. of Mice Exposed	No. of Mice Infected	% Transmission	Range in Virus Titers of Mosquitoes Following Infectious Meal*
$10^{-6.6}$	0						
	7	304	32	21	2	10	2.4–2.6
	9	399	80	27	9	33	2.2–4.0
	12	128	28	21	4	19	3.0–4.1
	14	158	33	28	13	46	2.9–4.2
	21	177	53	19	15	79	2.2–5.5
$10^{-4.8}$	0						
	7	125	20	10	1	10	3.4–5.1
	9	210	57	14	5	36	3.3
	12	261	118	18	13	72	2.6–5.5
	14	137	52	13	11	84	4.6–4.7
	21	47	12	8	6	75	5.3
$10^{-2.8}$	0						
	7	72	23	8	0	0	3.2
	9	66	12	7	0	0	2.0
	12	74	22	7	0	0	1.5
	14	93	10	7	0	0	1.8
	21	56	21	7	0	0	2.5

* Negative logarithm.

periods. Since great variations in titer, sometimes amounting to two logs or more, were obtained in different experiments with an identical incubation period, it is difficult to correlate these findings with the results obtained by transmission. However the data suggest that there exists a minimum titer of approximately $10^{-2.2}$ in the mosqui'o below which transmission is infrequent. This point is brought out in more detail later in the discussion of experiments with individual mosquitoes.

Relationship of dose of virus fed mosquitoes and their ability to transmit infection. The results of transmission from Table 1 indicated that, at an extrinsic incubation period of 14–21 days, transmission of virus by bite to infant mice occurs with regularity and with high frequency. Since, under the laboratory conditions employed, the mortality of the mosquitoes was high on the 21st day of the extrinsic incubation period, day 14 was chosen for all subsequent experiments, with the exception of those where the length of the incubation period was investigated. Table 2 summarizes the results of transmission of West Nile virus to infant mice after such a 14-day incubation period. When the titers of the suspensions fed to mosquitoes were between $10^{-4.0}$ and $10^{-6.6}$ a high percentage of transmission, in many instances up to 100 per cent, was obtained, whereas,

TABLE 2

Titer of virus fed to mosquitoes, and titer found after an extrinsic incubation period of 14 days as related to the transmission of virus to infant mice

Negative Log of Titer of Suspension Fed to Mosquitoes	No. of Infected Mosquitoes to Which Infant Mice Were Exposed	No. of Mosquitoes Which Fed on Mice	Ratio of Mice Infected to Mice Exposed	Titer of Virus in Mosquitoes on 14th Day[a]
6.6	64	14	7/7	4.2
6.6	16	5	2/5	3.6
6.6	34	9	3/8	3.7
5.5	75	29	4/6	3.6
5.4	155	55	7/7	5.7
5.2	122	36	6/6	3.5
5.0	76	26	6/7	3.5
5.0	71	18	5/5	3.6
4.3	84	34	6/7	4.6
4.3	53	18	5/6	4.7
4.0	80	23	3/5	3.4
2.8	86	21	0/8	1.0
2.8	98	5	0/7	2.4
2.8	96	5	0/6	2.5
2.0	72	20	0/6	

when the suspensions fed had titers between $10^{-2.0}$ and $10^{-2.8}$, no transmission occurred. The titers of virus found in the mosquitoes after an incubation period of 14 days were generally lower when the smaller doses of virus had been fed to the insects.

The length of the extrinsic incubation period. In a few cases, attempts were made to investigate mosquito transmission of infection before the 7th and after the 21st day. The titers of virus found in the mosquitoes on these days were routinely measured. Unfortunately, it was impossible to induce significant numbers of mosquitoes to bite infant mice until the seventh day following the infectious meal. On the other hand, it was difficult to secure a large number of mosquitoes after the 28th day since natural mortality exceeded 95 per cent by that time. Table 3 gives the results of these experiments. It will be seen that already at the first hour after feeding, the titer of virus in the mosquitoes was lower than that of the suspension fed. The titer dropped further on days one and three of the extrinsic incubation period, but virus remained detectable at all times, and rose again by the end of one week. In two instances, a sufficient number of mosquitoes was secured through the 28th day, and when allowed to bite infant mice, they were still able to transmit the infection to 9 of 15 animals.

Dose of virus transmitted by infected mosquitoes. The amount of virus injected into infant mice by the bite of infected mosquitoes was approximated as follows. The percentage of infant mice coming down with encephalitis on different days following the infectious bite was compared with that of mice coming down after subcutaneous inoculation of graded amounts of virus. The subcutaneous route was chosen for the purpose of comparison since it seems to be the closest to the "natural" one. All experiments in which mice came down with signs of CNS

TABLE 3

Titer of virus in mosquitoes and its transmission to infant mice after short and long extrinsic incubation periods

Negative Log of Titer of Suspension Fed to Mosquitoes	Time After Infectious Meal	No. of Infected Mosquitoes to Which Mice Were Exposed	No. of Mosquitoes Which Fed on Mice	Ratio of Mice Infected to Mice Exposed	Titer of Virus in Mosquitoes
5.5	0				
	1 hour	75			2.7
	1 day	101			<1.0
	3 days	63			<1.0
	7 days	27	12	0/6	1.2
	14 days	75	29	4/6	3.6
	28 days	69	10	9/15	3.7
4.3	0				
	1 hour	56			3.0
	7 days	103	18	1/4	3.4
	9 days	134	34	5/7	
	12 days	44	9	4/7	2.6
	14 days	53	18	5/6	4.7

involvement following the bite of infected mosquitoes, were combined and are tabulated in Table 4 in decreasing order of titers of suspensions fed to mosquitoes. It will be seen that no mice came down during the first three days following the bite of infective mosquitoes; half of the mice came down on day 4; and about 94 per cent through the sixth day. In Fig. 1, the cumulative percentages of mice coming down after subcutaneous inoculation of different doses of West Nile virus or the bite of infected mosquitoes, are plotted against days following inoculation or bite. The figure shows that the curve for the mosquito bite falls very close to that obtained by the subcutaneous inoculation of 1000 ID_{50} of virus, which indicates that this dose of virus was injected by the bite of infected mosquitoes.

Transmission of infection by single mosquitoes to individual mice. In the experiments so far reported in this paper, pools of mosquitoes were used for the determination of the titers of virus in the mosquitoes and "massive" biting was employed, using on the average 3–6 mosquitoes per infant mouse. Although this method enabled us to perform large scale experiments in relatively short periods of time, it yielded no information on individual variations in infectivity of single mosquitoes and on the transmission of virus to mice by the bite of single mosquitoes. Thus, many problems on the dynamics of infection and transmission were left unsolved among which were the following:

(a) Does each mosquito that feeds on a virus suspension get infected?

(b) What is the titer of virus in a single mosquito? Is an infected mosquito pool composed of insects having uniform titers or does it contain mosquitoes with relatively high titers and others with no virus at all?

(c) Is the bite of a single infected mosquito sufficient to convey the infection to an infant mouse?

TABLE 4

Intrinsic incubation period in infant mice following bites of mosquitoes infected with West Nile virus

Titer of Suspensions Fed to Mosquitoes	Extrinsic Incubation Period in Days	No. of Mice Bitten	Mice Showing CNS Involvement; Day Following Infectious Bite														Ratio of Mice Infected to Mice Exposed
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	
6.6*	7	8	—	—	—	—	1	1	—	—	—	—	—	—	—	—	2/8
6.6	9	7	—	—	—	3	2	1	—	—	—	—	—	—	—	—	6/7
6.6	9	8	—	—	—	1	1	1	—	—	—	—	—	—	—	—	3/8
6.6	12	7	—	—	—	2	—	—	—	1	—	—	1	—	—	—	4/7
6.6	14	8	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1/8
6.6	14	8	—	—	—	1	—	—	—	—	2	—	—	—	—	—	3/8
6.6	14	7	—	—	—	7											7/7
6.6	14	5	—	—	—	2	—	—	—	—	—	—	—	—	—	—	2/5
6.6	21	7	—	—	—	4	3										7/7
6.6	21	8	—	—	—	7	1										8/8
5.5	14	6	—	—	—	4	—	—	—	—	—	—	—	—	—	—	4/6
5.5	28	7	—	—	—	3	1	—	—	—	—	—	—	—	—	—	4/7
5.5	28	8	—	—	—	2	2	—	—	1	—	—	—	—	—	—	5/8
5.2	14	6	—	—	—	3	2	1									6/6
5.0	14	4	—	—	—	4											4/4
5.0	14	7	—	—	—	1	3	2	—	—	—	—	—	—	—	—	6/7
5.0	14	5	—	—	—	—	1	3	1								5/5
4.4	10	6	—	—	—	—	1	—	—	—	—	—	—	—	—	—	1/6
4.4	14	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1/2
4.3	7	4	—	—	—	—	—	1	—	—	—	—	—	—	—	—	1/4
4.3	9	7	—	—	—	—	4	1	—	—	—	—	—	—	—	—	5/7
4.3	12	7	—	—	—	5	1	—	—	—	—	—	—	—	—	—	6/7
4.3	12	4	—	—	—	—	2	1	—	—	—	—	—	—	—	—	3/4
4.3	12	7	—	—	—	—	2	2	—	—	—	—	—	—	—	—	4/7
4.3	14	7	—	—	—	6	—	—	—	—	—	—	—	—	—	—	6/7
4.3	14	6	—	—	—	—	4	—	1	—	—	—	—	—	—	—	5/6
4.3	21	8	—	—	—	2	4	—	—	—	—	—	—	—	—	—	6/8
4.0	14	5	—	—	—	2	—	1	—	—	—	—	—	—	—	—	3/5
4.0–6.6	7–28	179	—	—	—	61	35	15	2	2	2	—	1	—	—	—	118/179

* In negative logs.

(d) Does any relation exist between the titer of virus in a mosquito and its ability to transmit the virus by bite?

To answer these questions, experiments were performed in which single mosquitoes were fed upon marked infant mice. After biting, the titer of virus in each mosquito was determined separately and the fate of the bitten mouse

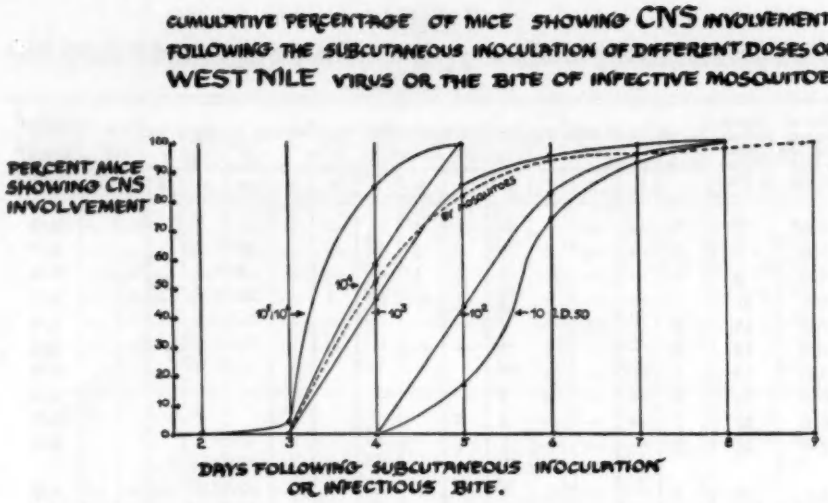
CUMULATIVE PERCENTAGE OF MICE SHOWING CNS INVOLVEMENT
FOLLOWING THE SUBCUTANEOUS INOCULATION OF DIFFERENT DOSES OF
WEST NILE VIRUS OR THE BITE OF INFECTIVE MOSQUITOES
PERCENT MICE SHOWING CNS INVOLVEMENT
BY MOSQUITOES
10^4
10^3
10^2
10 L.D.50
DAYS FOLLOWING SUBCUTANEOUS INOCULATION OR INFECTIOUS BITE.

FIG. 1. Between 54 and 77 infant mice were used at each dose of virus inoculated subcutaneously to obtain the curve for that dose.

TABLE 5

Transmission of virus to individual mice by the bite of single mosquitoes after an extrinsic incubation period of 14 days

No. of Mosquitoes	Titer of Each Mosquito after Biting Test Mouse	No. of Mice Infected / No. of Mice Bitten
4	<1.0, <1.0, 1.6, 1.7	0/4
8*	>1.5 for each	3/8
6	2.2, 2.2, 2.2, 2.5, 2.6, 2.7	3/6
1	3.5	1/1

* Titer of suspension fed to mosquitoes was $10^{-5.0}$ for each of these 8 insects, for the other 11 mosquitoes it was $10^{-6.6}$.

followed. Each mosquito which fed on a virus suspension having a titer of $10^{-5.0}$ or $10^{-6.6}$, contained virus after a period of 14 days. However, as shown in Table 5, individual mosquitoes had different virus titers ranging from less than 1.0 to 3.5. Not all the infected mosquitoes were able to transmit the infection to infant mice, though virus was still present in their bodies 14 days after feeding on a virus suspension. When the titer of virus in the mosquito was below $10^{-1.5}$, no transmission occurred. About 50 per cent of the mosquitoes were able to transmit the infection, when the titer of virus in their body exceeded $10^{-1.5}$. Although in some cases the titer of an individual mosquito was higher than $10^{-2.5}$, transmission did not always occur.

Similar experiments with individual mosquitoes were performed after an extrinsic incubation period of 21 days. It was found that seven out of ten mosquitoes which had fed on a virus suspension having a titer of $10^{-6.6}$ were able

TABLE 6

*Distribution of virus in the tissues of infant mice at different time intervals following the subcutaneous inoculation‡ of West Nile virus and the bite of infected mosquitoes**

Day Following Infectious Bite	Overt Illness	Titer of Virus in Tissue							
		Heart blood		Brain		Liver		Spleen	
		Inoculation	Bite	Inoculation	Bite	Inoculation	Bite	Inoculation	Bite
1	None	2.3	2.7	<1.0	<1.0	<1.0	1.3	<1.0	1.6
2	None	5.5	3.5	5.2	5.9	2.8	3.2	1.5	3.4
3	None	5.5	5.1	7.6	7.3	4.5	3.6	4.3	3.9
4†	Encephalitis	6.3	5.2	8.5	7.4	6.0	3.3	5.6	3.7

* Titer of suspension fed to mosquitoes = $10^{-6.6}$.

† No animal remained well after the 4th day.

‡ Each mouse was inoculated subcutaneously with 320 ID_{50} of virus.

to transmit the infection to infant mice. This seems to indicate that the infection rate of mosquitoes is higher on day 21, i.e., 70 per cent, than on day 14 when it was 37 per cent.

Distribution of virus in infant mice following the bite of an infected mosquito. For determining the location of virus in different tissues after the "natural" route of infection, infant mice were bitten by infected mosquitoes. The procedure of such an experiment was as follows. A few hundred mosquitoes were fed on a suspension of virus having a titer of $10^{-6.6}$. After an extrinsic incubation period of 14 days, about 150 of these mosquitoes were allowed to feed on a group of 24 two-day old infant mice. After three hours feeding, about 50 per cent of the mosquitoes were found to be engorged. This represents an average of 3–4 mosquito bites per mouse. At intervals of 24 hours, starting one day after infection, groups of 4 mice were sacrificed and their heart-bloods, brains, livers and spleens pooled respectively. The blood clots and organs were triturated and the suspensions obtained were titrated intracerebrally in 3–4 week old mice. The remaining infant mice all came down with definite signs of encephalitis and died on the 4th and 5th days. The results of a representative experiment are shown in Table 6, and compared with those obtained by the subcutaneous inoculation of West Nile virus in infant mice. On the first day following the bite of infective mosquitoes, the titer of the virus was highest in the blood. On the following days, the titer of virus in the blood rose, but this seems to be proportionate to the high titer of virus in the brain, beginning on the second day. The virus titer of the brain reached a maximum on the third day, although no overt illness was as yet manifest in the mouse. The virus titers of the liver and spleen were low on the first day, attained their maximal height after 48 hours, and remained static thereafter.

According to previous calculations (*vide supra*), it was estimated that the dose of virus injected by mosquitoes is in the vicinity of 1000 ID_{50}. Therefore, for comparison purposes, it was aimed to inoculate a similar dose of virus. However, titrations of the virus suspension inoculated revealed that 320 ID_{50} were

actually inoculated. It will be seen from Table 6 that the titers of virus in the tissues of infant mice following the subcutaneous inoculation of 320 ID_{50} of virus were very similar to those following the bite of infective mosquitoes.

The fate of virus in the different tissues of infant mice following the bite of infected mosquitoes was found to be similar to the distribution of virus after subcutaneous inoculation.

DISCUSSION

Certain points emerge from the results of this study which cover several aspects of infection of *Culex molestus* by West Nile virus and transmission of the virus by bite to susceptible animals. These pertain to:

a) The amount of virus necessary to infect the mosquitoes and enable them to transmit this infection to infant mice.

b) The level of virus attained in the body of the mosquito at different time intervals following the infective meal, its relation to the amount of virus ingested by the mosquitoes and to their ability to transmit the virus by bite.

c) The length of the extrinsic incubation period and the duration of infectivity of the mosquitoes.

d) Dosage and fate of virus injected by the mosquito into the susceptible animal.

It is of importance, especially for the guidance of the epidemiologist, to know the dosage of virus necessary to infect the insect vector so that transmission can occur. The results of the experiments reported indicate that for transmission of West Nile virus to take place, the titer of the infective suspension ingested by the mosquito must exceed $10^{-2.8}$ (calculated in terms of 0.03 ml. of suspension—the amount inoculated intracerebrally into mice). When fed on suspensions below this "threshold" titer, mosquitoes did not transmit the virus to infant mice although virus could be demonstrated in the insect bodies by inoculation intracerebrally into mice. It was unimportant whether the titer of the suspension fed to mosquitoes was $10^{-4.0}$ or $10^{-6.6}$; whenever the titer exceeded $10^{-4.0}$, transmission occurred with regularity after a suitable incubation period. These data are in accord with the results of studies on transmission of the virus of yellow fever by mosquitoes (Whitman, 1937; Kumm and Laemmert, 1950; Bates and Roca Garcia, 1946). These authors investigated the infection of the mosquitoes by feeding on monkeys having varying titers of virus in their blood. With blood levels of virus below 1000 ID_{50}, infection of the mosquitoes was infrequent, whereas a high percentage of mosquitoes became infected when the titer of virus in the blood exceeded 10,000 ID_{50}.

The finding that the titer of the virus in the mosquito body drops considerably during the first few days following the infective meal is in agreement with the results obtained by Whitman (1937) for the virus of yellow fever, and by Kitaoka (1950) and Davies and Yoshpe-Purer (1954) for West Nile virus. Even one hour after the infective meal, the titer of virus recovered from the ground mosquito bodies was considerably less than that of the suspension fed. The titer of virus in the mosquitoes rose between the 3rd and 7th day and continued to

increase gradually thereafter until it reached a level somewhat higher than that of the suspension fed, indicating that multiplication of virus occurred in the insect. The titers of virus attained between the 9th and 28th days were almost of the same magnitude but varied considerably in different experiments. However, when the suspension fed had a titer of $10^{-2.8}$, the titer of virus in the mosquito pool was definitely lower than with suspensions having titers of $10^{-4.0}$ or higher. On these days of the incubation period, the titers of virus in the mosquitoes fluctuated between $10^{-2.2}$ and $10^{-5.7}$. A similar range of titers in mosquitoes infected with West Nile virus was obtained by Philip and Smadel (1943), and Kitaoka (1950). It has been reported that in *Aëdes aegypti* (Davies and Yoshpe-Purer, 1954) the titers of virus in the mosquitoes attain much higher levels, reaching a titer of over $10^{-9.0}$ on the 21st day of the incubation period.

No direct relationship was observed between the height of the titer of virus in the mosquitoes and the results of transmission of the virus to infant mice by the bite of these mosquitoes. Often, different pools of mosquitoes possessing similar titers of virus transmitted the infection at varying rates. However, experiments in which individual mosquitoes were fed upon single infant mice are easier to interpret. When mosquitoes fed on virus suspensions having titers of $10^{-5.0}$ and $10^{-6.6}$, each one became infected. The titers of virus in the individual mosquitoes varied over a range of less than $10^{-1.0}$ to $10^{-3.5}$, pointing to individual differences. The results also indicate that in the individual mosquito a certain "threshold" titer is necessary for transmission to occur, this threshold titer being around $10^{-1.5}$. However, not each mosquito that had a titer exceeding $10^{-1.5}$ necessarily transmitted the virus to infant mice. Only about half of them did so.

The experimental results show that transmission of virus to infant mice by the bite of infected mosquitoes occurred between the 7th and the 28th day following the infectious meal. It is probable that the extrinsic incubation period is shorter than 7 days but, unfortunately, no data are available since it was impossible to induce the mosquitoes to feed on infant mice before that day. Because very few mosquitoes remained alive for 28 days following the infective meal, no complete data are available as to the duration of the infectivity of the mosquitoes. The data on the length of the extrinsic incubation period and the duration of infectivity are in accord with the findings of others working on the transmission of West Nile virus by mosquitoes (Philip and Smadel, 1943; Kitaoka, 1950; Taylor and Hurlbut, 1953; Davies and Yoshpe-Purer, 1954). The last workers reported transmission of West Nile virus by *Aëdes aegypti* to last up to 38 days.

One of the problems that faced us was the determination of the amount of virus injected into an infant mouse by the bite of infected mosquitoes. It has recently been demonstrated by Griffiths and Gordon (1952) that mosquito saliva is introduced into the subcutaneous tissue of the mouse in the form of "puffs", before the mosquito feeds from a blood vessel. It was thought, therefore, that quantitative comparisons of the effects caused by the mosquito bites could be made with those caused by subcutaneous inoculation of measured amounts of virus. It is realized that the amount of virus injected by the mosquitoes was

arrived at in an indirect way; nevertheless the results seem to fit quite closely with those obtained for the virus of yellow fever and *Aëdes aegypti* (Davis, 1934) by direct determinations. The distribution of virus in infant mice following the bite of infective mosquitoes shows the same general trend as that obtained by the subcutaneous inoculation of virus.

SUMMARY

Culex molestus was infected by feeding on suspensions of West Nile virus and the virus transmitted to susceptible animals. Strains of virus recently isolated from the blood of West Nile fever patients in Israel were used. When the titer of the suspension fed was $10^{-4.0}$ or more, virus was transmitted by mosquitoes to infant mice after an extrinsic incubation period of 7–28 days. When the titer was $10^{-2.0}$ or $10^{-2.8}$, transmission did not occur, even though virus was present in the mosquitoes. The percentage of mice infected by bite seemed to be a function of the length of the extrinsic incubation period.

The titer of virus in infected mosquitoes varied considerably in different experiments and fluctuated between $10^{-2.2}$ and $10^{-5.7}$ between the 9th and 28th day of the incubation period. It was found that an infected mosquito pool may be composed of individual mosquitoes having titers of virus ranging from less than $10^{-1.0}$ to $10^{-3.5}$. Though each mosquito feeding on a suspension having a titer of $10^{-5.0}$ or more became infected, only half of those infected were able to transmit the infection by bite after a suitable incubation period. The amount of virus injected into infant mice by the bite of infected mosquitoes was found to correspond to about 1000 ID_{50}.

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COMPARISONS OF SULFISOXAZOLE WITH SULFADIAZINE, AND THIOCYMETIN WITH CHLORAMPHENICOL, IN CHEMOTHERAPY OF EXPERIMENTAL PLAGUE IN MICE

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In the prophylaxis of the infection caused by *Pasteurella pestis*, sulfadiazine (Sokhey *et al.*, 1952; Meyer *et al.*, 1952) has been found to be the drug of choice, and chloramphenicol (Smadel *et al.*, 1952, McCrumb *et al.*, 1953) has been shown suitable for therapeutic use by oral administration. It seemed desirable to compare new and potentially useful agents, especially those having a similar chemical configuration, with these medicinals.

In a clinical study, sulfisoxazole (3,4-dimethyl-5 sulfanilamidoisoxazole) has been found to give the least undesirable reactions in comparison with sulfapyridine, sulfathiazole, and sulfadiazine (Yow *et al.*, 1953). It has been shown to be effective in treatment of a number of bacterial infections. In addition, the greater solubility of sulfisoxazole over sulfadiazine led to the testing of this sulfa drug against the plague bacillus. Thiocymetin, a new synthetic antibacterial agent, is dd-threo-2-dichloroacetamido-1-(*p*-methylsulfonylphenyl)-1,3-propanediol, and differs from chloramphenicol in chemical configuration only in that the *para* position contains methyl-sulfonyl instead of a nitro group (Cutler *et al.*, 1952).

Since thiocymetin possesses a high antibacterial activity, (Dennis *et al.*, 1952), its action against *P. pestis* was compared with that of chloramphenicol *in vitro* by broth dilutions and *in vivo* in laboratory mice. Similarly, sulfisoxazole was compared with sulfadiazine.

METHODS

In vitro testing

Sterile 20 per cent solutions of sulfadiazine and of sulfisoxazole were made with distilled water and a sufficient volume of *N* sodium hydroxide was added to produce clear solutions. These solutions were sterilized by steam autoclaving and then diluted while warm with sterile brainheart-infusion broth to a concentration of 20 mg. per ml.

Solutions of chloramphenicol and of thiocymetin were made by dissolving 16 mg. in 6 ml. of alcohol and then adding sterile distilled water to make 10 ml. After these solutions had been kept overnight at room temperature, 1 to 10 dilutions of each were made with sterile broth. One ml. volumes of these broth solutions were aseptically transferred into the first 2 tubes of each series of sterile test tubes; and then, to each tube save the first in the series, was added one ml.

of sterile broth. Serial 2-fold dilutions were made beginning with the 2nd tube and excluding the penultimate tube of each series; the last tube was the control which contained only broth and no drug. The different series were then inoculated by adding to each test tube one ml. of 10^{-3} dilution of 2nd broth transfer of plague strains grown at 30°C. for 24 hours. Considering the experimental error, plate counts showed, for each of the strains used, 3×10^8 organisms per ml. of the original bacterial suspension. The broth drug dilutions were inoculated to contain 1.5×10^6 bacilli per ml. The tubes were shaken and then incubated at 37°C. After 24 and 48 hours the degree of growth was measured by turbidity and a loopful of the broth from each tube was streaked onto a section of a blood agar plate on the 2nd and 7th day to estimate the bactericidal effect. To test the effect of the sulfonamides on smaller inocula of *P. pestis*, 10^{-4} dilutions of the strains were used instead of 10^{-3} concentrations in one ml. quantities.

In vivo tests

Swiss laboratory mice (20–32 gm.) were infected by intraperitoneal injection of 0.5 ml. doses of either 10^{-4} or 10^{-6} dilutions of a second transfer in broth of virulent plague bacilli (Alexander strain, human case, N. Mexico). These inocula represented 1,000 and 10 LD_{50} doses, respectively. The mice were then mixed and divided into weight groups of 10 each for treatment. The drug solutions were prepared by dissolving the amount necessary for 3 doses (360 mg.) in the minimal volume of alcohol (12 ml.) required for the least soluble drug (thiocymetin). The alcoholic solutions were equally divided and then diluted with an equal volume of glycerine before water was added in an amount necessary to give the dose in 0.4 ml. to a 20 gm. mouse. The drugs were administered by stomach tube; one dose at 1–3 hours after infection, another at 8–10 hours, and the last dose at 24–26 hours. The mice were observed for 10 days when all survivors were sacrificed.

RESULTS AND DISCUSSION

Effectiveness of sulfonamides against plague bacilli has been amply documented (Schütze, 1939; Girard & Girard, 1939; Sokhey *et al.*, 1940; Meyer *et al.*, 1952). Sulfadiazine (2-sulfanilamidopyrimidine) is one of the most active sulfa drugs. In comparison, however, sulfisoxazole has been found more active than sulfadiazine against all ten strains of plague used (see Table 1).

The difference in activity could not be attributed to difference in the molecular weights, since the weight ratio of the isoxazole compound to the diazine compound is small, being 267 to 250. It may, however, be due to the different solubilities of the two compounds and this property could be an important factor *in vitro*.

Bacteriostatic concentrations of antibiotics currently employed against *P. pestis* are about 1,000 times less than those necessary in the case of sulfonamides. With chloramphenicol, 2.5 micrograms have about the same effect as 2.5 milligrams of sulfadiazine. Thiocymetin in broth is about 10 to 20 times less potent

TABLE 1

Effect of sulfadiazine and of sulfisoxazole on the growth of P. pestis *in broth*

Plague Strain	Days Incubated	Concentration of Sulfadiazine														Concentration of Sulfisoxazole												
		Mg/cc for 10^{-3} inoculum							μg/cc for 10^{-4} inoculum							Mg/cc for 10^{-3} inoculum							μg/cc for 10^{-4} inoculum						
		10	5.0	2.5	1.3	0.6	0.3	0.0	40	20	10	5.0	2.5	1.3	0.0	10	5.0	2.5	1.3	0.6	0.3	0.0	40	20	10	5.0	2.5	1.3	0.0
195/P	1	p	p	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	‡	‡	+	0	0	0	0	0	0	0
	2	(+)	(++)	(+++)	(++)	(++)	(++)	(++)	(15)	(50)	(33)	(+)	(++)	(++)	(++)	(0)	(10)	(+)	(++)	‡	‡	(++)	(0)	(0)	(2)	(20)	(+)	(++)	(++)
	7	(+)	(+)	(+++)	(++)	(++)	(++)	(++)	(0)	(++)	(++)	(++)	(++)	(++)	(++)	(0)	(7)	(6)	(50)	‡	‡		(0)	(0)	(0)	(++)	(++)	(++)	(++)
Alexander	1	p	p	+	+	‡	‡	+	0	0	0	0	0	0	±	+	+	+	+	‡	‡	‡	0	0	0	0	0	0	0
	2	(35)	(+)	(+++)	(++)			(++)	(0)	(0)	(7)	(+)	(++)	(++)	(++)	(2)	(8)	(30)	(+)	‡	‡	‡	(0)	(0)	(0)	(1)	(++)	(++)	(++)
	7	(3)	(+)	(+++)					(0)	(0)	(+)					(0)	(0)	(0)	(50)				(0)	(0)	(3)				
Yreka	1	p	p	+	+	‡	‡	+	0	0	0				0	+	+	+	+	‡	‡	‡	0	0	0				0
	2	(+)	(+)	(+++)	(++)			(++)	(0)	(0)	(25)				(++)	(0)	(7)	(30)	(+)				(1)	(3)	(0)				(++)
	7	(80)	(+)	(+++)					(0)	(0)	(++)					(0)	(0)	(20)	(40)				(0)	(0)	(0)				
Wash. 53J2	1	p	p	+	+	‡	‡	+	0	0	0				0	+	+	+	+	‡	‡	‡	0	0	0				0
	2	(40)	(++)	(+++)	(++)			(++)	(1)	(0)	(50)				(++)	(1)	(6)	(15)	(+)				(5)	(0)	(0)				(++)
	7	(10)	(++)	(+++)					(0)	(c)	(++)					(0)	(4)	(20)	(40)				(0)	(0)	(0)				
N. Mexico 53M12	1	p	p	+	+	‡	‡	+								+	+	+	+	‡	‡	+							
	2	(20)	(+)	(+++)	(++)			(++)								(4)	(10)	(60)	(+)										
	7	(4)	(+)	(+++)												(4)	(10)	(50)	(80)			(++)							
Calif. 53A25	1	p	p	+	+	‡	‡	+	0	0	0				+	+	+	+	+	‡	‡	‡							
	2	(1)	(++)	(++)	(++)			(++)	(1)	(1)	(0)				(++)	(1)	(8)	(20)	(+)				0	0	0				+
	7	(0)	(+)	(+)					(0)	(0)	(+)					(2)	(0)	(7)	(20)				(0)	(1)	(1)				(++)
A1122	1	p	p	+	+	‡	‡	+	0	0	0				+	+	+	+	+	‡	‡	‡							
	2	(5)	(+)	(+++)	(++)			(++)	(0)	(3)	(1)				(++)	(8)	(50)	(+)	(++)				0	0	0				+
	7	(0)	(+)	(+++)					(0)	(0)	(+)					(0)	(0)	(3)	(20)				(0)	(2)	(0)				(++)
EV-76 (1951)	1	p	p	+	+	‡	‡	+	0	0	0	0	0	0	0	+	+	+	+	‡	‡	‡	0	0	0	0	0	0	0
	2	(15)	(+)	(+)	(+)			(++)	(0)	(0)	(7)	(+)	(++)	(++)	(++)	(0)	(2)	(8)	(+)	‡	‡	‡	(0)	(0)	(0)	(1)	(++)	(++)	(++)
	7	(50)	(+)	(++)					(1)	(10)	(+)					(0)	(0)	(0)	(0)				(0)	(6)	(50)				
14	1	p	p	+	+	‡	‡	+	0	0	0	0	0	+	+	+	+	+	+	‡	‡	‡	0	0	0	0	0	0	+
	2	(10)	(+)	(+++)	(++)			(++)	(0)	(0)	(+)	(+)	(+)	(++)	(++)	(50)	(+)	(++)	(++)	‡	‡	‡	(2)	(7)	(0)	(25)	(++)	(++)	(++)
	7	(0)	(+)	(+++)					(0)	(2)	(++)	(++)	(++)			(0)	(0)	(7)	(50)				(0)	(0)	(1)				
Hawaii S-113	1	p	p	+	+	‡	‡	+								+	+	+	+	‡	‡	+							
	2	(2)	(+)	(+++)	(++)			(++)								(5)	(20)	(60)	+										
	7	(0)	(+)	(+++)												(0)	(0)	(4)	(4)			(++)							

p—precipitate; +—good growth; c—contaminated; (0)—reading of streak on agar plate, no colonies of plague; (++)—confluent heavy growth; ±—doubtful growth.

than chloramphenicol as a bacteriostatic agent against the 10 strains of plague (Table 2). In this pair of compounds the differences in molecular weight are as small as in the pair of sulfonamides.

Thus the *in vitro* activity of these 4 compounds in descending order is; chloramphenicol, thiocymetin, sulfisoxazole, and sulfadiazine.

The 3-methyl analog of dimethyl sulfisoxazole has been shown to be less active than sulfadiazine (Anderson *et al.*, 1942; Bell & Roblin, 1942; Backer & de Jonge, 1943), whereas the biological activity of the compound without any methyl substitution has not been tested. Apropos of substitution of methyl groups in the N^1 heterocyclic ring, the activity of the derivatives of sulfisoxazole differs from that of sulfadiazine. Thus the dimethylpyrimidine, sulfamethazine, is neither as active as the monomethylpyrimidine, sulfamerazine, nor sulfadiazine, which has no methyl substituent in the pyrimidine nucleus (Frisk, 1943). For comparing the antibacterial activity due to lesser structural differences, the analogs of sulfamilamido-thiazole and oxazole are better since they differ from each other only by one atom belonging to the same electron group, oxygen and sulfur. Unfortunately, not many analogous pairs have been synthesized and studied for antibacterial properties. The 2-sulfanilamido-thiazole (sulfathiazole) and 2-sulfanilamido-oxazole are effective, with sulfathiazole having the greater potency (Anderson *et al.*, 1942; Bell & Roblin, 1942). However, when the 5-position is substituted with a benzene-ring, the oxazale compound appears to be more active (Jensen, 1942; Kharasch & Reinmuth, 1943). The 5-sulfanilamido-thiazole is bacteriostatic to streptococcus but has no advantages over the 2-derivative, sulfathiazole (Arnold & Scaife, 1944). The 5 sulfanilamido-isoxazole has not been studied. This discussion of structural relationship to antibacterial activity, although indirect for sulfisoxazole, is sufficient to demonstrate that both sulfadiazine and sulfisoxazole are superior compounds among their analogs.

Similar to the sulfanilamide type of compound, the chemical configuration responsible for the antibacterial action of chloramphenicol has been established (Controulis *et al.*, 1949; Feitelson *et al.*, 1951). While it has been found that certain structural characteristics of the chloramphenicol molecule could not be at all altered without drastic sacrifice of antibacterial activity, e.g., the optical isomers possess but little activity (Maxwell & Nickel, 1954), other properties, such as substitution of various radicals for the nitro group, do not bias the activity (Collins *et al.*, 1952). Derivatives with substituents in the *para* phenyl position place of the nitro group of chloramphenicol have been reported bacteriostatic (Buu-Hoi *et al.*, 1950; Cutler *et al.*, 1952). Thus the high activity of the *para* methylsulfonylphenyl analog of chloramphenicol is not unusual. Within the more specific portion of the molecule, the requirements were so fulfilled that the L-isomer of thiocymetin is completely inactive. Biologically, the action and potency of thiocymetin are so similar to chloramphenicol as to suggest that both act by the same mechanism.

In practice, either due to economy or availability, the baseline of acceptable biological activity for sulfonamides is about 1,000-fold less than that for antibiotics. Chloramphenicol in this study (Tables 1, 2) is about 500 times as active as

TABLE 2

Effect of thiocymetin and chloramphenicol on growth of P. pestis *in broth*

	Day of Test	Concentration of Thiocymetin (microgram/cc)							Concentration of Chloramphenicol (microgram/cc)							
		80	40	20	10	5	2.5	0.00	80	40	20	10	5	2.5	1.25	0.62
195/P	1	0	0	0	0	+	+	+	0	0	0	0	0	0	+	+
	2	0(+)	0(7)	+(++)	+(++)	+(++)	+(++)	+(++)	0(50)	0(50)	0(50)	0(30)	0(10)	0(28)	+	+
	7	+(++)	+(++)	+	+	+	+	+(++)	0(1)	0(9)	9(11)	+(++)	+(++)	+(++)	(++)	
Alexander	1	0	+	+	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(+)	+(++)	+(++)	+	+	+	+(++)	0(34)	0(35)	0(35)	0(15)	0(5)	+(70)	+	+
	7	+(++)	+(++)	+	+	+	+	+(++)	0(2)	0(6)	0(7)	+(+)	+(++)	+(++)	(++)	
Yreka	1	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(40)	0(8)	+(+)	+(++)	+	+	+(++)	0(17)	0(10)	0(30)	0(12)	0(1)	+(25)	+	+
	7	+(++)	+(++)	+(++)	+	+	+	+(++)	0(1)	0(2)	0(8)	0(11)	+(++)	+(++)	(++)	
Wash. 53J2	1	0	0	0	±	+	+	+	0	0	0	0	0	0	+	+
	2	0(14)	0(9)	0(80)	±(++)	+(++)	+(++)	+(++)	0(12)	0(8)	0(75)	0(9)	0(2)	0(+)	+	+
	7	+(++)	+(++)	+(++)	+	+	+	+(++)	0(0)	0(3)	0(27)	+(0)	+(++)	+(++)	(++)	
N. Mex. 53M12	1	0	0	0	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(8)	0(2)	0(++)	+(++)	+(++)	+	+(++)	0(27)	0(8)	0(35)	0(5)	0(0)	+(60)	+	+
	7	+(++)	+(++)	+	+	+	+	+(++)	0(0)	+(1)	+(11)	+(3)	+(+)	+(++)	(++)	
Calif. 53A25	1	0	0	0	±	+	+	+	0	0	0	0	0	0	+	+
	2	0(25)	0(35)	+(++)	+(++)	+(++)	+	+(++)	0(12)	0(3)	0(0)	0(6)	0(1)	0(+)	+	+
	7	+(++)	+(++)	+	+	+	+	+(++)	0(0)	0(0)	0(1)	+(0)	+(+)	+(++)	(++)	
A1122	1	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(20)	0(+)	+(++)	+	+	+	+(++)	0(12)	0(8)	0(50)	0(35)	0(3)	+(++)	+	+
	7	+(++)	+(++)	+(++)	+	+	+	+(++)	0(0)	0(1)	0(35)	+(17)	+(++)	+(++)	(++)	
EV-76	1	0	0	0	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(+)	0(80)	0(+)	+(++)	+(++)	+(++)	+(++)	0(+)	0(+)	0(+)	0(+)	0(35)	0(+)	+	+
	7	+(++)	+(++)	+(++)	+	+	+	+(++)	0(13)	0(22)	0(31)	+(80)	+(++)	+(++)	(++)	
14	1	0	0	+	+	+	+	+	0	0	0	0	0	0	+	+
	2	0(25)	+(++)	+(++)	+(++)	+	+	+(++)	0(+)	0(12)	0(35)	0(35)	0(0)	+(++)	+	+
	7	+(++)	+	+	+	+	+	+(++)	0(1)	0(5)	0(12)	+(7)	+(++)	+(++)	(++)	
Hawaii S-113	1	0	0	0	+	+	+	+	0	0	0	0	0	0	0	+
	2	0(60)	0(14)	0(+)	+(++)	+(++)	+(++)	+(++)	0(70)	0(20)	0(20)	0(40)	0(9)	0(0)	±	+
	7	+(++)	+(++)	+(++)	+	+	+	+(++)	0(8)	0(1)	0(10)	0(37)	+(50)	+(++)	+	

Readings as in Table 1.
Figures in parenthesis indicate number of colonies which developed from a loopful streaked onto a section of blood agar, +—too many colonies to count, ++—confluent growth.

TABLE 3

Therapeutic effect of chloramphenicol, thiocymetin, sulfadiazine, and Gantrisin on plague-infected mice

Compound Chemical	Dose* Mg./Kg.	1,000 LD_{50}								10 LD_{50}										
		Days							% surv.	Days										% surv.
		1	2	3	4	5	6	7		1	2	3	4	5	6	7	8	9	10	
Chloramphenicol	100	10	10	10	3	0	0	0	0	10	10	10	10	10	10	10	10	10	10	100
	50	10	10	9	1	0	0	0	0	10	10	10	10	8	8	8	8	8	8	80
	25	10	10	3	0	0	0	0	0	10	10	10	9	9	9	7	7	6	5	50
	12.5	10	9	0	0	0	0	0	0	10	10	8	2	2	2	1	1	1	1	10
Thiocymetin	100	10	10	5	0	0	0	0	0	10	10	6	5	4	4	4	4	4	4	40
	50	10	7	0	0	0	0	0	0	10	10	8	6	6	5	5	4	4	4	40
	25	10	7	0	0	0	0	0	0	10	10	10	9	7	5	3	3	3	3	30
	12.5	10	10	4	2	1	1	0	0	10	10	6	4	5	3	3	3	3	3	30
Sulfadiazine	100	10	10	10	7	4	2	1	10	10	10	10	10	7	7	6	5	5	5	50
	50	10	10	10	10	3	0	0	0	10	10	10	3	1	1	1	1	1	1	10
	25	—	—	—	—	—	—	—	—	10	10	10	7	6	5	5	5	5	5	50
	12.5	10	10	6	0	0	0	0	0	10	10	10	8	3	2	2	2	2	2	20
Gantrisin	100	10	10	7	0	0	0	0	0	10	10	9	3	3	3	3	3	3	3	30
	50	10	10	2	0	0	0	0	0	10	10	9	4	2	2	2	2	2	1	10
	25	10	6	1	0	0	0	0	0	10	10	8	3	1	0	0	0	0	0	0
	12.5	10	4	0	0	0	0	0	0	10	10	6	4	4	3	2	0	0	0	20
Water	1×10^6	9	3	0	0	0	0	0	0	10	9	5	1	1	0	0	0	0	0	0
None		10	4	1	1	0	0	0	0	10	10	8	5	5	5	4	4	4	4	40

* Each dosage concentration was administered orally 3 times, i.e., respectively at 1, 8 and 24 hours, after intraperitoneal inoculation.

sulfisoxazole and 1,000 to 5,000 times as active as sulfadiazine. Actually, the mechanism of action of these synthetic antibiotics and the sulfonamides is different. In the case of a sulfanilamide type of compound, the antibacterial activity has been explained as a competitive inhibition of a growth factor, *para* aminobenzoic acid (Woods, 1940; Fildes, 1940), whereas chloramphenicol inhibits specific enzymes, e.g., diamine oxidase (Zeller *et al.*, 1949; Hahn & Wisseman, 1951). Since thiocymetin acts similarly to chloramphenicol, a direct enzyme inhibiting mechanism would account for its greater *in vitro* activity as compared to that of the sulfonamides.

The lower activity (Table 3) *in vivo* as compared to that *in vitro* has been explained through the binding of sulfonamide molecules by protein molecules (Anderson *et al.*, 1942). This hypothesis, however, would not account for the *in vivo* action of sulfadiazine and sulfathiazole which are bound by proteins in higher percentage than sulfanilamide and sulfapyridine (Davis, 1943). A better explanation for the lower *in vivo* action of sulfisoxazole is found in its higher excretion rate. This same reason has been given to account for the lower potential of sulfathiazole in comparison with sulfadiazine in the chemotherapy of plague (Quan, 1944; Meyer *et al.*, 1952). Since urine anlyses have indicated that thiocymetin is rapidly excreted by the kidneys (Dennis, 1954), the lower therapeutic value shown by this compound in the study in comparison with chloramphenicol is also better explained on this basis than by either a degradation (Smith *et al.*, 1949; Berti, 1950) or a more rapid reduction (Glazko *et al.*, 1949 & 1952).

SUMMARY

In broth cultures of 10 strains of *Pasteurella pestis*, 3,4-dimethyl-5-sulfanilamido-isoxazole (Gantrisin, sulfisoxazole) in concentrations of 8 to 16 times less than that of 2-sulfanilamidopyrimidine (sulfadiazine) produced a similar degree of bacteriostasis. Against the same strains of plague, chloramphenicol was 16 to 32 times more inhibitory than thiocymetin. Chloramphenicol exerted a marked antibacterial action at a concentration of 2.5 microgram/ml. as compared with about the same action for 1.3 mg./ml. of sulfisoxazole. Thus *in vitro* chloramphenicol was about 20 times more potent than thiocymetin, 500 times more than sulfisoxazole, and 5,000 times more than sulfadiazine. Since the mechanism of action of the synthetic antibiotics differs from that of the sulfonamides, the two types of drugs are not comparable. This difference is illustrated by the fact that sulfadiazine proved to be the most effective among these chemicals for experimental plague prophylaxis in laboratory mice, whereas chloramphenicol was the best therapeutic agent. The *in vivo* order of efficacy was chloramphenicol, sulfadiazine, thiocymetin, and sulfisoxazole. The lower therapeutic value of sulfisoxazole compared with sulfadiazine, and of thiocymetin compared with chloramphenicol is probably due to more rapid excretion by the kidneys.

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A BRIEF SURVEY OF INTESTINAL PARASITES OF MAN IN THE HASHEMITE KINGDOM OF JORDAN*

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This brief study on intestinal parasites among the inhabitants of Jerusalem and Amman, Jordan, is part of the work carried out in 1954 by the senior author during an assignment to the Central Government Laboratory in Amman. A record of it is desirable because there is little published information about parasitic infection in that locality.

DESCRIPTION OF THE AREA

During recent conflicts in Palestine, thousands of Palestinian Arabs have sought refuge in Jerusalem and Amman and in surrounding areas. Some refugees have their homes and places of business in the cities, but many live in tents. The sudden change, and crowded, poor conditions have brought about deterioration in the hygienic and sanitary habits of the people.

The Arab sector of Jerusalem has a population of about 47,000 persons, with about 83,000 refugees in the vicinity. The city, located 13 miles west of the northern end of the Dead Sea, has an altitude of approximately 2,600 feet. Amman, a relatively new city, is situated 45 miles northeast of Jerusalem at an altitude of about 2,500 feet. When made the capital of the country about 30 years ago, Amman was a very small village. It now has an estimated population of 140,000 of which at least 80,000 are refugees. Jerusalem, as well as Amman, is surrounded by arid farm lands. The climate in both cities is fairly cold in winter with frost and occasional snow. The summers are dry with the temperature rarely reaching 90°F. Rain usually falls between the end of October and April or May. There is drought from June to September. The average annual rainfall in Jerusalem is about 21 inches and in Amman about 13 inches. The relative annual humidity in Jerusalem is believed to be higher than Amman since humidity and rainfall in the area generally decrease from north to south and from west to east. The predominating winds throughout the year are westerly or south-westerly, the latter being most marked in winter with the rains. Easterly or south-easterly winds from the desert are most common from April to early June and from September to November.

The economic level of a large portion of the people of the area, as well as of their sanitary habits and practices, is generally low. Flies are abundant during the warmer months. They have easy access to the interiors of houses, and public

* Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 340.

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and private latrines which are generally unkempt. Foodstuff, including bread, fruits and vegetables, is often displayed on streets or in front of stores without protection from dust and flies. It is well known that some of the vegetables commonly eaten raw are grown in soil irrigated with water containing human excreta. Food is frequently handled and eaten with unclean hands. Due to the general scarcity, water in suburban areas and villages is frequently obtained from open cisterns or springs and stored in open receptacles in homes. Under such conditions abundant opportunity exists for contamination and transmission of bacterial, protozoal and helminthic infections.

MATERIALS AND METHODS

Four hundred and twenty-five persons were examined in this study. Of these, 125 were from Jerusalem, and 300 from Amman. The stool samples were collected from various sources including government and private hospitals, schools and government employees. The hospitalized individuals did not include those who were suffering from an acute or chronic intestinal trouble. Only one normal stool was obtained from each person and sent to the laboratory in a standard sputum cup not later than a few hours after passing. The stools were first examined by the direct smear method, one unstained in saline and one stained with iodine (modified Dobell's). About 1 gram of each stool was then examined on the same day by the concentration method using the formalin-ether technique as given by Ritchie (1948). Samples of stool specimens which were not examined on the same day, were first screened and washed in physiological saline by centrifugation, and then the sediment was mixed and stored in 7.5 per cent formalin. This formalized material was later examined by the formalin-ether method, usually within one or two days.

FINDINGS AND DISCUSSION

The pertinent findings in this study, based upon a single fecal specimen from each individual, are given in Tables 1 and 2. No attempt has been made to segregate the data into age groups since the number of persons examined in each area is comparatively small. The percentage of infection is given for the two sexes; however, the data is actually not sufficient to be of real significance for comparing the two groups. The percentage of parasitism recorded for Jerusalem and Amman cannot be considered comparable because the survey in Jerusalem was conducted during the spring of the year, and in Amman during the summer months. Undoubtedly chance of parasite transmission and infection is less common in the cooler months than in the warmer months.

The most important result brought out in this survey is the finding that *Entamoeba histolytica*, *Ascaris lumbricoides* and *Trichuris trichiura* are the most important intestinal pathogenic parasites in the area. Small and large races of *E. histolytica* cysts were seen. The incidence of *E. histolytica* and other protozoa in Jerusalem was found to be much lower than that found in Amman. As already pointed out, this difference might have been due, in part, to the time of the year that the two studies were conducted. In Jerusalem during the month of April, the incidence of *E. histolytica* was 12 per cent, whereas in Amman, where the

TABLE 1

Incidence of intestinal parasitic infection in Jerusalem. Study conducted during the month of April, 1954

Parasites	Number of Positive				Total Males and Females	
	Males		Females			
	No.	Per cent	No.	Per cent	No.	Per cent
Entamoeba histolytica	12	16.7	3	5.7	15	12.0
Entamoeba coli	22	30.6	18	34.0	40	32.0
Endolimax nana	13	18.1	5	9.5	18	14.4
Iodamoeba bütschlii	6	8.3	2	3.7	8	6.4
Chilomastix mesnili	4	5.6	1	1.9	5	4.0
Giardia lamblia	3	4.1	2	3.7	5	4.0
Trichomonas hominis	3	4.1	4	7.5	7	6.4
Ascaris lumbricoides	54	75.0	43	81.1	97	77.6
Trichuris trichiura	55	76.4	43	81.1	98	78.4
Strongyloides stercoralis	2	2.8			2	1.6
Trichostrongylus sp.	1	1.4			1	0.8
Taenia sp.	2	2.8	1	1.9	3	2.4
Hymenolepis nana			2	3.7	2	1.6
Total examined	72		53		125	

TABLE 2

Incidence of intestinal parasitic infection in Amman. Study conducted during the months of August to October, 1954

Parasites	Number of Positive				Total Males and Females	
	Males		Females			
	No.	Per cent	No.	Per cent	No.	Per cent
Entamoeba histolytica	48	23.1	13	13.9	61	20.3
Entamoeba coli	76	36.7	38	40.9	114	38.0
Endolimax nana	72	34.8	34	36.4	106	35.3
Iodamoeba bütschlii	48	23.1	15	16.1	63	21.0
Chilomastix mesnili	52	25.1	41	44.1	93	31.0
Giardia lamblia	18	8.7	7	7.5	25	8.3
Trichomonas hominis	14	6.8	6	6.5	20	6.7
Ascaris lumbricoides	92	44.4	62	66.7	154	51.3
Trichuris trichiura	92	44.4	41	44.1	133	44.3
Strongyloides stercoralis	1	0.5			1	0.3
Trichostrongylus sp.	1	0.5			1	0.3
Taenia sp.	4	1.9			4	1.3
Hymenolepis nana	5	24			5	1.7
Total examined	207		93		300	

study was conducted from August to October, the per cent of *E. histolytica* was 20.3. The fact that the time of the year bears some relationship to the incidence of *E. histolytica*, and possibly to other protozoa, is indicated in a nine months' record (February–September 1954) of stool examinations at the Augusta Vic-

toria Hospital in Jerusalem (Canaan, 1954). According to this record, the following percentage of *E. histolytica* was found (the number in parentheses refers to number of patients examined): February, 7.3 per cent (304); March, 7.7 per cent (312); April, 9.1 per cent (439); May, 8.6 per cent (443); June, 16.3 per cent (417); July, 16.9 per cent (413); August, 18.7 per cent (487); September, 22.2 per cent (528). These data show a definite increase of *E. histolytica* during the summer months.

The incidence of *E. histolytica* among the male population, both in Jerusalem and Amman, was somewhat higher than among the females. The question of sex differences in parasitic infection is not well understood and probably varies in different localities depending on the peculiar habits or practices of the people. Eyles *et al.* (1953) in a study of intestinal parasites in a rural West Tennessee community found a higher incidence of parasitism among the females. On the other hand Svensson (1935) in a study among a Swedish group, concluded that in an urban community the males tended to have a higher rate of parasitism. *Ascaris lumbricoides* and *Trichuris trichiura* were the most prevalent and widespread helminthic infections. The incidence of these two parasites in Jerusalem was uniform, 77.6 per cent for ascarids and 78.4 per cent for trichurids. In Amman, the incidence although lower, was also somewhat uniform, 51.3 per cent for ascarids and 44.3 per cent for trichurids. The prevalence of these parasites is undoubtedly due to the resistance of the eggs to adverse conditions and their ability to develop under a wide range of environment (Cort, 1931). Climatic conditions are probably a factor in the lower incidence of these parasites in Amman. As already indicated, rainfall and humidity are lower in Amman than in Jerusalem, and therefore less suitable for the survival of the parasite eggs.

In the spread of protozoan and helminthic infections in nature, houseflies in all probability play an important role. They are known to ingest and excrete ameba cysts and helminth eggs (Wenyon *et al.*, 1917). Furthermore, viable ameba cysts have been found in fly droppings at least 48 hours after an infective feed (Root, 1921).

Considering the generally poor sanitary conditions or habits and closeness of group and family associations characteristic of Arabic countries, the incidence of parasitism, especially of the protozoa, as found in this survey is comparatively low. This finding is similar to a survey of intestinal parasites of Yemen, Southwest Arabia, by Kuntz *et al.* (1953). However, the incidence of parasitism in this study would have been higher if more stool specimens had been examined from each individual. Svensson (1935), working with identification of protozoa parasites, reported that at least six or more stools are required to reveal a true percentage of infection in a population. In connection with the incidence of parasites in this area, however, the presence of warm temperature, intense sunlight, and drought for about four months of the year, must exert considerable detrimental effect on the viability of many parasitic eggs and cysts.

No large scale study was conducted to determine the incidence of *Enterobius vermicularis*. Eggs of this parasite were encountered only twice in all of the 425 stools examined. However, in the examination made in Jerusalem of 26 individ-

uals between 5 and 15 years of age using the NIH swab method of Hall (1937), 5, or 19.2 per cent showed the eggs of this parasite. Of interest in this survey was the apparent absence or rareness of hookworm infection. Possibly this is due to the lack of soil moisture and intense sunlight over several months of the year which make unsuitable the completion of the extra human phases of the life cycle of the parasite.

SUMMARY

A single stool specimen from each of 125 individuals in Jerusalem and 300 in Amman was examined for the purpose of determining the type and incidence of intestinal parasitism.

The percentage prevalence of the most important pathogenic parasites was: *Entamoeba histolytica*, 12.0 per cent in Jerusalem and 20.3 per cent in Amman; *Ascaris lumbricoides*, 77.6 per cent in Jerusalem and 51.3 per cent in Amman; *Trichuris trichiura*, 78 per cent in Jerusalem and 44.3 per cent in Amman. The indicated incidence of infection between these two cities is not considered comparable because the survey in Jerusalem was conducted during the cooler months of the year, and in Amman during the warmer months when chance of parasite transmission is probably greater.

Considering the prevalence of unsanitary conditions, abundance of houseflies during the summer months, and the close association of the population, the incidence of intestinal parasitism in these areas is considered to be comparatively low. Intense sunlight, drought, and dryness of the soil are probably important factors in the destruction of many eggs and cysts of parasites.

Acknowledgement is made to Mr. Farah Musharbash for assistance in the examination of some of the fecal specimens.

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EPIDEMIOLOGICAL STUDY ON SCHISTOSOMIASIS JAPONICA IN FORMOSA[1]

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In a historical review of the epidemiology of schistosomiasis japonica in Formosa (Hsü *et al.*, 1952), it was pointed out that there has been only meager reference to the disease on this island. Most books on parasitology or tropical medicine state that endemic schistosomiasis japonica is present in Formosa. However, a review of the literature indicates that authentic indigenous human cases of this disease have never been found there, although *Schistosoma japonicum* has been reported in domestic animals (Takegami, 1914, 1915 and Takegami *et al.*, 1914) as well as in the intermediate host, *Oncomelania formosana* (Yokogawa, 1915). The specimens were identified as *S. japonicum* by Yokogawa (1915) and Faust and Meleney (1924).

In order to have a better understanding of the epidemiology of this disease in Formosa, work was started in 1950 on the distribution and incidence of *S. japonicum* infection in the intermediate host, *O. formosana*, and on the incidence of its infection in domestic animals and small wild mammals in the Changhua endemic area. These studies showed that the average incidence of infection of *Oncomelania* snails collected from contaminated ditches was 1.3 per cent with a range from 0.3 to 30 per cent (Hsü *et al.*, 1952). In the domestic animals examined, 62 per cent of dogs, 3.5 per cent of water buffaloes, 2.6 per cent of pigs and 1.8 per cent of goats were infected (Hsü, Hsü and Chu, 1954). In the small wild mammals, the incidence of infection was 8.6 per cent for *Rattus rattus*, 21.5 per cent for *R. norvegicus*, 16.4 per cent for *R. losea*, 12.5 per cent for *Mus formosanus*, and 18.6 per cent for *Crocidura murina* (Hsü and Li, in manuscript).

With high incidence of *S. japonicum* infection in the intermediate host and in so-called reservoir hosts, one would expect a high incidence of human cases of schistosomiasis japonica in this area. In an attempt to solve the problem, an extensive search for human cases of schistosomiasis japonica was done in 1952 and the results of the investigation are presented in this paper.

METHODS

Stool examination. Stool examinations were done in the seven villages where incidence of *S. japonicum* in *Oncomelania* snails had been found high (Hsü *et al.*, 1952) and the inhabitants had frequent opportunities to come in contact with water in the snail-harboring ditches. In these villages, three stool examinations were done on each person five years of age or more.

[1] This investigation was supported by research grants, E. −228 (C), T. M. (1), and E. −939, P. T. M. (1), National Microbiological Institute, of the National Institutes of Health, United States Public Health Service.

[2] Medical Zoology Department, 406th Medical General Laboratory, U. S. Army, taking part in the intradermal test.

Intradermal test. Using antigen prepared from the adult worms of *S. japonicum* obtained from Japan, the intradermal test was performed on the inhabitants in six villages where examinations had been done. The antigen was proved potent by making a preliminary test on three Chinese in Formosa who acquired their *S. japonicum* infection while on the Chinese mainland. About 0.01 ml. of the merthiolated antigen (1:10,000 dilution) and a merthiolated saline control were injected into the volar surface of the forearm. Reading of the test was done 15 minutes after injection and an increase of 3 mm. in the diameter of the wheal as compared with the control was considered a positive reaction. In order to rule out the possibility that the positive reaction might be caused by hypersensitivity to other factors, inhabitants in a village outside the endemic area were tested with the same antigen.

Re-examination of stools from persons with positive intradermal reactions. Three additional stools were obtained from each person showing a positive intradermal reaction, and each was examined by three methods, e.g., sedimentation, acid-ether, and hatching.

RESULTS OF EXAMINATION

Stool examination. Seven villages, Tayu, Wayao and Niuputso in Puyen *hsiang* and Tingliao, Peishihwei, Chutien and Fanpo in Chihu *chen*, were chosen for examination (for map, see Fig 6, Hsü *et al.*, 1952). From these villages, 11,952 stools from 3,984 persons were examined. In addition, 639 stools from 213 persons were examined from other villages in the endemic area, making a total of 12,591 stools from 4,197 persons. In each of three persons, one doubtful *S. japonicum* egg was found. The contour of these eggs was not typical and the miracidium could not be visualized. The examination of six additional stools from each of these persons failed to show additional eggs. All other stools were negative for *S. japonicum*.

Intradermal test. The intradermal test was done on 2,562 persons from 6 villages in the endemic area, Tayu, Wayao, Niuputso, Peishihwei, Chutien and Fanpo (Table 1), and 142 persons from the village of Shihku in a non-endemic area. The proportion of persons in the endemic area showing positive reactions

TABLE 1

Results of intradermal tests for schistosomiasis japonica in the Changhua endemic area, Formosa

Village	No. Examined	No. Positive	Percentage Positive
Tayu	695	49	7.1
Wayao	473	37	7.8
Niuputso	430	67	15.6
Peishihwei	443	25	5.6
Chutien	261	21	8.0
Fanpo	260	15	5.8
Total	2,562	214	8.4 (average)

TABLE 2

Results of intradermal tests for schistosomiasis japonica in the Changhua endemic area, tabulated according to age and sex of the persons tested

Age in Years	Number Examined			Number Positive			Percentage Positive		
	M. & F.	Male	Female	M. & F.	Male	Female	M. & F.	Male	Female
3–7	92	48	44	0	0	0	0	0	0
8–12	532	255	277	6	5	1	1.1	2.0	0.4
13–17	504	260	244	40	27	13	7.9	10.4	5.3
18–22	298	149	149	38	24	14	12.8	16.1	9.4
23–27	227	101	126	19	12	7	8.4	11.9	5.6
28–32	190	89	101	24	23	1	12.6	25.8	1.0
33–37	148	70	78	21	17	4	14.2	24.3	5.1
38–42	147	59	88	19	15	4	12.9	25.4	4.5
43–47	128	52	76	11	10	1	8.6	19.2	1.3
48–52	110	48	62	10	7	3	9.1	14.6	4.8
53–57	65	31	34	10	9	1	15.4	29.0	2.9
58–62	43	21	22	4	4	0	9.3	19.0	0
63–67	38	19	19	9	9	0	23.7	47.4	0
68–72	24	14	10	2	2	0	8.3	14.3	0
73–77	14	7	7	1	1	0	7.1	14.3	0
78–82	2	2	0	0	0	0	0	0	0
Total.....	2,562	1,225	1,337	214	165	49	8.35	13.5	3.7

averaged 8.4 (range, 5.6 to 15.6) whereas all persons (99 males and 43 females, age 7 to 61) from the non-endemic village were negative to the test.[3] The 3 persons with a doubtful *S. japonicum* egg in the stool were negative to the intradermal test.

The results of the intradermal tests were tabulated according to age and sex of the persons tested (Table 2). Statistical analysis using the chi square test was made for the sex and age prevalence of the positive reactors. The results of the analysis indicate: (1) that the portion of positive reactors is significantly less for women than for men; (2) that there appears to be a tendency for females to have a positive reaction during ages 13 through 57 but not to the same extent as for males; (3) that the portion of positive reactors is significantly less for the age group 3 through 12 for the combined group and for males than for the age group 13 to 82 (the limit of the data); and (4) that no significant difference was found in the portion of positive reactors among the higher age groups for either the combined group or for males.

Re-examination of stools from persons with positive intradermal reactions. Three stools from each of the 214 persons showing positive intradermal reactions were re-examined. Three techniques were used on each sample (sedimentation, acid-ether and hatching). All samples were again negative for *S. japonicum* eggs.

[3] There was one doubtful reactor in this group, but as in the village of the endemic area, such reactors were considered negative.

DISCUSSION

The results obtained from the examination of 13,233[4] human stools, collected in the villages in Formosa most likely to have *S. japonicum* infections, indicate that there are no indigenous human cases with mature, egg-producing worms. In the same area, the persons making these examinations and using the same techniques had no difficulty in finding the eggs in the stools of wild and domestic animals. It is possible that three atypical eggs were seen in human samples. Six additional examinations failed to show eggs in the stools of these three persons. It has been shown that *S. japonicum* eggs are present in the livers of pigs and goats from this area (Hsü, Hsü and Chu, 1954). These are often eaten so the three questionable eggs may have come from ingested liver.

It may appear that the results obtained in the intradermal tests contradict those reported for the stool examinations. In the endemic area, 8.4 per cent of persons tested gave positive reactions, while those outside it were negative. These results indicate the type of infection found in Formosa, rather than throwing doubt on the data from the stool examinations. It has been shown that some other species of schistosomes can invade the lungs, the liver or the mesenteric veins and live there for certain periods of time in an abnormal host without producing eggs (Fairley, 1927; Penner, 1941; Olivier, 1949; Kagan, 1953) and it has also been known that some persons infected with non-human schistosome cercariae give a positive reaction to the intradermal test (Cort, 1936; Macfarlane, 1949). The persons giving positive reactions in the present study can reasonably be assumed to have acquired their sensitivity from infections of this sort. This hypothesis has been verified in human volunteers (Hsü and Hsü, in manuscript). All the information available suggests that the *S. japonicum* in Formosa is a zoophilic strain which uses domestic and certain small, wild mammals as natural definitive hosts.

Because of this unusual relationship it is of interest to compare the data of intradermal reaction obtained in Formosa with those reported from areas where the human strain is present. In Chuhsien, China, Kan (1936) reported positive reactions in 77.9 per cent of the general population. At Kurume, Japan, Inoue *et al.* (1942) found that the positives ranged from 12.1 to 59.2 per cent in school children (ages, approximately 7 to 14). Only 8.4 per cent of the general population and 3.6 per cent of the school children of the same ages gave positive intradermal reactions in Formosa. From an epidemiological point of view, it is of interest that the incidence of infection in the molluscan intermediate hosts in the various areas is about the same regardless of the strain of parasite. On the other hand, Kan found the positive reactions higher in males than females, 81.7 vs 26.3 per cent, Inoue *et al.* obtained the same result from the school children (14.5 to 71.4 vs 9.2 to 43 per cent), and in Formosa a similar relationship was found, with 13.5 per cent in males and 3.7 per cent in females. This can easily be explained by the fact that in the Orient, females usually do not

[4] 12,591 stool samples were examined previous to, and 642 after, the performance of the intradermal test.

work in the fields as frequently as males and therefore have less opportunity to come in contact with the cercariae-harboring water in irrigation ditches.

With regard to age prevalence, we cannot find suitable data of intradermal reaction from the human-strain areas for comparison (Kan did not classify his data according to age groups). However, some investigators have studied the age prevalence of *S. japonicum* infection in human-strain areas from their results of stool examinations. Tubangui and Pasco (1941) reported from their results of stool examinations at Naujan, Mindoro, P. I., that infection percentage was lowest in children of both sexes who were under 10 years of age, highest in males between 11 and 30 years of age and in females between 11 and 20, and slightly lower and more or less uniform throughout in the older age group. Also at Naujan, Hunter *et al.* (1950) made a study of 209 unselected individuals over 5 years of age with sexes evenly divided and reported that the incidence increased rapidly during the first two decades of life, showed a slow rise during the third decade, and developed a peak in the fourth decade, after which it dropped abruptly. In a word, previous investigators have shown that the incidence of infection is greatest in the second, third and fourth decades of life. Results from the intradermal tests in Formosa show that positive reactors were significantly less in the 3 to 12 age groups, but significant differences were not found in the higher age groups.

Some of the apparent discrepancy in age incidence found in Formosa, as compared to endemic areas where a human strain is prevalent, may be due to the comparison of intradermal reactions in the one and stool examinations in the others. It is of interest, however, that in Iraq, Watson (1953) found no significant difference in the incidence of *S. haematobium* infection between the 5-year age group up to and over 65 years of age except for the group 15 to 19 years which showed a slightly higher figure. His results were obtained by urine examination. It will be of interest to make a further study on this subject as the results of such a study may throw light on the problem of age immunity against superinfection and reinfection of schistosomiasis as well as the difference in immunological manifestations between human and non-human schistosome strain infections.

SUMMARY

The incidence of *S. japonicum* in the molluscan intermediate host, and in various wild and domestic mammals was known to be relatively high in the endemic area in Formosa. The apparent absence of schistosome infection in the human population needed further study. Stool examinations by the sedimentation method were done in seven villages in the Changhua endemic area. An examination of 12,591 stools from 4,197 persons did not reveal a definite positive case of *S. japonicum* infection. The intradermal test for schistosomiasis japonica on 2,562 persons in six villages where stool examinations had been done showed 8.4 per cent of positive reactors. The incidence of positive reactions was significantly less for women than for men. It was also significantly less for the age group 3 through 12 than for the higher age groups but there was no significant difference among the higher age groups.

Three stools were obtained from each person giving a positive reaction (214 persons) and re-examined for *S. japonicum* infection by the sedimentation, acid-ether, and hatching methods. No positive cases were found in examination of these stools. An explanation has been given for the apparent discrepancy in the results obtained from stool examinations and intradermal tests. All the evidence obtained in this study indicates that *S. japonicum* in Formosa is a non-human, zoophilic strain.

ACKNOWLEDGEMENT

We wish to express our sincere appreciation to Dr. T. Hsiang Wang, Director, Chinese National Institute of Health, Taipei, Formosa, for his interest and assistance during the time when we were working in Formosa; to Messrs. T. C. Huang, C. T. Tsai and Y. W. Wang, Technical Experts of the same institute, for their technical assistance; and to Dr. Lloyd A. Knowler, Professor and Chairman of the Department of Mathematics and Astronomy, State University of Iowa, for his advice and assistance in making statistical analysis of the data as given in the present paper.

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TREATMENT OF VESICAL SCHISTOSOMIASIS WITH STIBOPHEN

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The ideal drug for the treatment of schistosomiasis has not yet been developed. Of those available, the trivalent antimonial stibophen (Repodrol, Fuadin) is one of the more satisfactory because it can be administered intramuscularly, it has a specific action on the parasites, and in general the untoward reactions are not too severe. However, the relapse rate is high, usually an estimated 50–55 per cent in *Schistosoma haematobium* infections. (Expert Committee on Bilharziasis, 1953.)

The present investigation had as its purpose a comparison of the results of various stibophen treatment schedules in vesical schistosomiasis in order to determine the most efficient method of administering this drug.

MATERIALS AND METHODS

Patients studied were plantation workers, all adult males, infected with *S. haematobium*, but living in a schistosome-free area with no opportunity for re-infection. The following five treatment schedules with intramuscular stibophen were tested:

Schedule A. 4 cc. on alternate days for 10 injections; total dosage 40 cc. administered over 20 days.

Schedule B. 5 cc. each day for four consecutive days, repeated after a treatment-free interval of four days; total dosage 40 cc. administered over 12 days.

Schedule C. 5 cc. the first day, 6 cc. the second and 7 cc. the third, with a six-day treatment-free period followed by 7 cc. and 8 cc. on two consecutive days; total dosage, 33 cc. administered over 11 days.

Schedule D. Drug was injected on each of two successive days and repeated after a treatment-free period of two to four days. Treatment was completed in six to eight days. Dosages were based on the patient's weight and were calculated thus: 140 pounds or over: 10 cc. each dose; total dosage 40 cc. 120–139 pounds: 9 cc. each dose; total dosage 36 cc. 100–119 pounds: 8 cc. each dose; total dosage 32 cc. 80–99 pounds: 7 cc. each dose; total dosage 28 cc.

Schedule E. Daily injections on each of three successive days, and dosage calculated on a weight basis as in Schedule D. Depending on the weight of the patients, total dosages varied from 21 cc. to 30 cc. and treatment was completed in three days.

All patients were ambulatory throughout the course of treatment.

The effectiveness of treatment was determined in each case by urine examinations which were continued whenever possible for six months following the completion of treatment. Urine specimens were collected and allowed to sediment, the supernatant fluid decanted and the remainder centrifuged. The entire sedi-

ment was then searched microscopically for living or degenerate *S. haematobium* eggs. Urines from each patient were examined an average of three times each month. The reappearance of living eggs in the urine was considered evidence of relapse. A total of 207 patients were treated and 114 of these were available for a six-month post-treatment study.

RESULTS

Untoward reactions. These included anorexia, nausea, vomiting, and a maculopapular pruritic skin eruption. The frequency with which they occurred in patients receiving the different treatment schedules is shown in Table 1. In addition to the above, a number of patients complained of weakness and generalized body pains following drug injections, but it was difficult to determine whether these complaints were legitimate or were actually reactions caused by the drug. These latter complaints were made by patients in all groups, but only in groups receiving treatment Schedules D and E were they considered of possible significance. Among Schedule E patients over 25 per cent lost from one to three days work, apparently as a result of the treatment.

TABLE 1

Untoward reactions in patients infected with vesical schistosomiasis and treated with intramuscular stibophen according to five different treatment schedules (see text)

Treatment Schedule	Number Treated	Nausea		Vomiting		Skin Eruption	
		Number	%	Number	%	Number	%
Schedule A.....	34	1	3	0	0	0	0
Schedule B.....	22	4	18	0	0	1	5
Schedule C.....	29	1	3	6	20	0	0
Schedule D.....	106	7	7	68	64	12	11
Schedule E.....	16	0	0	11	70	1	6

TABLE 2

Monthly post-treatment urine findings in adults infected with vesical schistosomiasis and treated with intramuscular injections of stibophen as follows: 4 cc. on alternate days for 10 injections; total dosage 40 cc. in 20 days

Treatment Schedule A

Number of Months Post-treatment	Number Treated	Living Eggs		Dead Eggs		Negative for Eggs	
		Number	%	Number	%	Number	%
1	27	0	0	11	40	16	60
2	22	0	0	7	32	15	68
3	19	2	11	5	26	12	63
4	19	2	11	3	16	14	73
5	13	3	23	4	30	6	47
6	12	4	33	2	17	6	50

TABLE 3

Monthly post-treatment urine findings in adults infected with vesical schistosomiasis and treated with intra-muscular injections of stibophen as follows: 5 cc. on each of four consecutive days, repeated after a treatment-free interval of 4 days; total dosage 40 cc. in 12 days

Treatment Schedule B

Number of Months Post-treatment	Number Treated	Living Eggs		Dead Eggs		Negative for Eggs	
		Number	%	Number	%	Number	%
1	14	0	0	8	57	6	43
2	14	0	0	6	43	8	57
3	9	0	0	1	11	8	89
4	9	1	11	1	11	7	78
5	9	1	11	3	33	5	56
6	9	1	11	2	22	6	67

TABLE 4

Monthly post-treatment urine findings in adults infected with vesical schistosomiasis and treated with intra-muscular injections of stibophen as follows: 5 cc. the first day, 6 cc. the second and 7 cc. the third, with a six-day treatment-free period followed by 7 cc. and 8 cc. on two consecutive days; total dosage 33 cc. in 11 days

Treatment Schedule C

Number of Months Post-treatment	Number Treated	Living Eggs		Dead Eggs		Negative for Eggs	
		Number	%	Number	%	Number	%
1	27	0	0	6	22	21	78
2	22	1	5	2	9	19	86
3	22	2	9	1	5	19	86
4	19	2	11	2	11	15	78
5	18	2	11	1	6	15	83
6	18	3	16	1	6	14	78

TABLE 5

Monthly post-treatment urine findings in adults infected with vesical schistosomiasis and treated with intramuscular injections of stibophen as follows: 7–10 cc. (depending on weight) daily for two consecutive days and repeated after a treatment-free period of two to four days; total dosage 28–40 cc. in 6–8 days

Treatment Schedule D

Number of Months Post-treatment	Number Treated	Living Eggs		Dead Eggs		Negative for Eggs	
		Number	%	Number	%	Number	%
1	88	3	4	34	39	51	57
2	88	7	9	27	30	54	61
3	78	9	11	16	21	53	68
4	74	9	12	11	15	54	73
5	72	10	14	7	10	55	76
6	71	10	14	5	7	56	79

TABLE 6

Monthly post-treatment urine findings in adults infected with vesical schistosomiasis and treated with intramuscular injections of stibophen as follows: 7–10 cc. (depending on weight) daily for three consecutive days; total dosage 21–30 cc. in three days

Treatment Schedule E

Number of Months Post-treatment	Number Treated	Living Eggs		Dead Eggs		Negative for Eggs	
		Number	%	Number	%	Number	%
1	—	—	—	—	—	—	—
2	6	1		2		3	50
3	4	2	50	0		2	50
4	4	2	50	0		2	50
5	4	2	50	0		2	50
6	4	2	50	0		2	50

Urine findings. The results of post-treatment urine examinations each month for all treated subjects are presented in Tables 2–6 and Figures 1 and 2. These show that the cure rate, based on the clearance of all schistosome eggs from the urine by the sixth post-treatment month ranged from 50 per cent to 79 per cent; Schedules A and E showed the poorest and Schedules C and D the best results.

DISCUSSION

The frequency and severity of untoward reactions seen in the treated patients showed a positive correlation with the size of the individual dose, but not necessarily with the total dosage given. Thus in Schedule A, where 40 cc. were given over a period of 20 days, reactions were negligible, but in Schedule E where patients received from 21 cc. to 30 cc. within three days, about 70 per cent vomited. However, vomiting was not usually considered a reason for interrupting treatment and did not materially affect the ability of the subject to continue with his work. The skin erruptions were never severe and did not interfere with completion of treatment; invariably they quickly disappeared after treatment was completed. However, it was difficult to evaluate the complaints of weakness and generalized pains attributed by some patients to treatment with stibophen. In Schedule E patients these complaints did appear to cause absenteeism from work and interference with completion of treatment in some cases. Such difficulties were encountered only rarely in patients receiving Schedule D treatment, and even less often in the other groups.

An analysis of urine findings in treated patients from the first to the sixth month post-treatment showed this drug to have an early specific action on the parasites and, with rare exceptions, urine specimens during the first post-treatment month were either negative or showed only degenerate eggs. However, by the fourth month post-treatment, some patients in all groups had relapsed as evidenced by the re-appearance of living eggs in their urine. New relapses appeared in each of the six post-treatment months but the interval of time elapsing after the end of treatment and before relapses occurred, appeared to depend on the intensity of the treatment schedule. Our results indicated that with approxi-

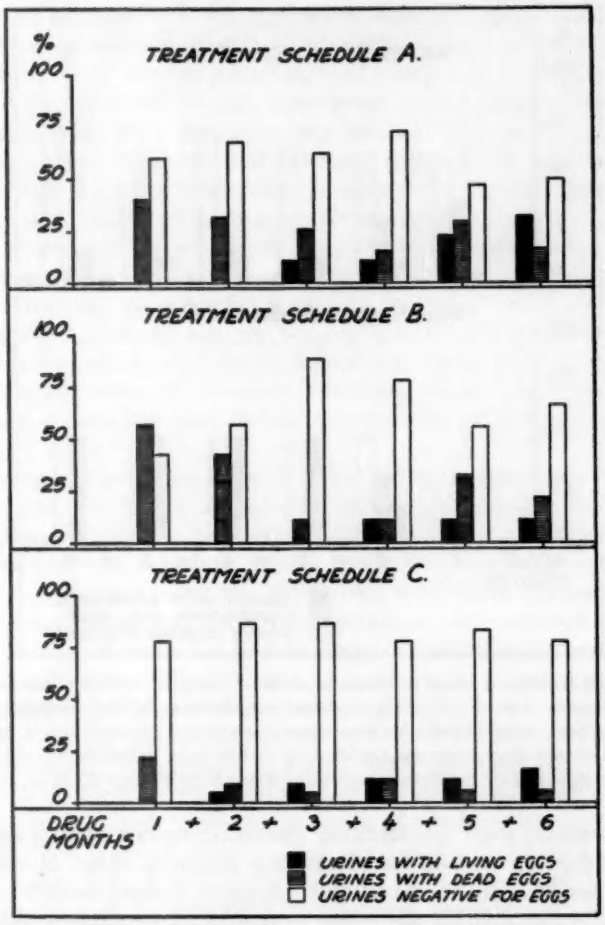

FIG. 1. Post-treatment urine findings in cases of vesical schistosomiasis with intramuscular stibophen according to three treatment schedules as follows: Schedule A: 4 cc. on alternate days for 10 injections; total dosage 40 cc. in 20 days. Schedule B: 5 cc. on each of four consecutive days, repeated after a treatment-free interval of 4 days; total dosage 40 cc. in 12 days. Schedule C: 5 cc. the first day, 6 cc. the second, 7 cc. the third, with a six day treatment-free period followed by 7 cc. and 8 cc. respectively on two consecutive days; total dosage 33 cc. in 11 days.

mately the same total dosage, more concentrated treatment regimes resulted in the earlier appearance of relapses. Groups receiving Schedules A and B, where the drug was given for 12 and 20 days respectively, did not show relapses until the third and fourth months post-treatment. The group receiving Schedule D, where treatment was completed in six to eight days, showed relapses during the first month post-treatment. These data suggest that in drug treatment trials

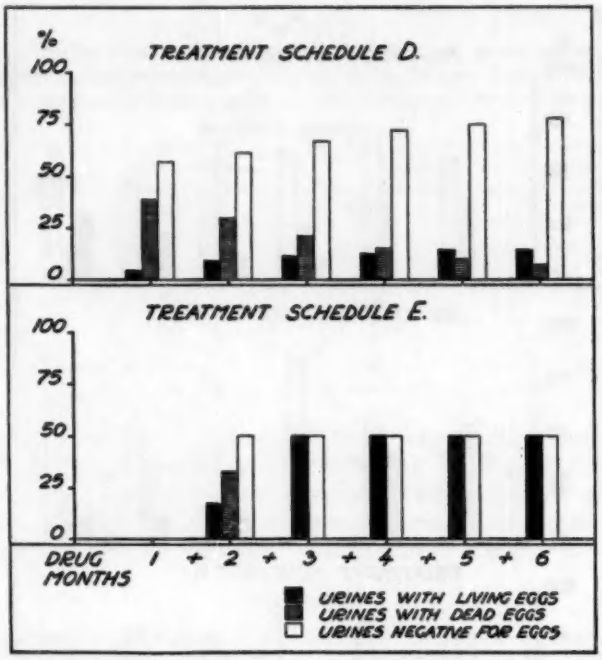

FIG. 2. Post-treatment urine findings in cases of vesicular schistosomiasis with intramuscular stibophen according to two treatment schedules as follows: Schedule D: 7–10 cc. (depending on body weight) daily for two consecutive days, repeated after a treatment-free period of two to four days; total dosage 28–40 cc. in 6–8 days. Schedule E: 7–10 cc. (depending on body weight) daily for three consecutive days; total dosage 21–30 cc. in three days.

for schistosomiasis, more concentrated treatment regimes require shorter post-treatment follow-up periods for discovering relapsing cases. However, in all instances it would appear to be advisable to study treated cases for a period of not less than six months for the proper evaluation of therapeutic efficiency.

The generally accepted criterion of relapse in treated cases of vesical schistosomiasis has been the re-appearance in the urine of living eggs. The significance of the presence of degenerate eggs in post-treatment urine specimens has not been established clearly and their continued presence in the urine under certain circumstances may well indicate unsuccessful treatment. It is a fact that during and following completion of treatment an increasing percentage of the eggs will be degenerate. Subsequently, as shown by our experiments, the urine findings will follow one of several patterns: (1) Degenerate eggs cease to be present after varying periods of time and the urine remains consistently negative for eggs; this is considered to be a cured case. (2) Degenerate eggs cease to be present, but subsequently on several occasions a rare degenerate egg is seen in the urine; this is considered to be a cured case, but with delayed tissue-passage of a few eggs. (3) Degenerate eggs cease to be present, but after a variable interval they

re-appear to be followed by living eggs; this is considered to be a relapsed case. (4) Degenerate eggs are present in the urine continuously through the six-month post-treatment. In (4) the following two possibilities may be considered. First, the worms were killed but a large reservoir of eggs being gradually extruded from the tissues explained the continuous presence of degenerate eggs in the urine. Secondly, the worms were not killed but their reproductive capacity was impaired in such a way as temporarily or permanently to interfere with their ability to produce healthy eggs. It is our belief, based on analyses of individual cases, that the continued presence in the urine of degenerate eggs over a six-month post-treatment period is more likely to indicate unsuccessful treatment in patients receiving an extended course of treatment with low individual dosages than it is with patients receiving a more intensive treatment regime. It is of interest to note that in the group receiving Schedule D treatment we had two cases originally showing very heavy infections, whose urine turned negative after degenerate eggs had been present continuously for four and five months respectively.

With respect to the comparative efficiency of the stibophen treatment schedules tested here, our results indicate that with approximately the same total dosage, higher cure rates can be expected with more intensive treatment regimes. Thus the results with Schedule B were better than with Schedule A, while Schedules C and D gave better results than did Schedule B. On the other hand, the total dosage given must also be considered, as demonstrated by the poor results in Schedule E where the highest drug concentration was employed, but where total dosages were 30 cc. or less.

Daily intramuscular injections of stibophen given for two successive days and repeated after two to four treatment-free days, as in Schedule D, proved to be relatively efficient in curing cases of vesical schistosomiasis (79%). Further, it was found to be the most convenient treatment regime to administer to large numbers in the field and, while the incidence of vomiting was high, untoward reactions did not interfere seriously with successful completion of treatment. Schedule D, therefore, is considered to be the treatment regime of choice for mass treatment. It is possible that by extending the treatment-free intervals to six days instead of two to four days as in the present experiment, the incidence and severity of untoward reactions could be reduced without impairing the efficiency of treatment.

A number of investigators have tested the effects of stibophen with various dosage schedules in the treatment of vesical schistosomiasis, but in practically all reported studies the results are to be viewed with reserve because of inadequate post-treatment study and/or the inability to exclude the possibility of re-infection. The study of Halawani and Dawood (1953) has taken these factors into consideration: their patients, being inmates of a reformatory, were not exposed to re-infection and they were studied for post-treatment periods of up to one year. They gave daily injections of stibophen (1 cc./12 kg. body weight) for periods ranging from 3 to 12 consecutive days, but considered the 12-day treatment course too toxic for general use. With the 10-day treatment their

cure rate (absence of living eggs in urine) at the end of six months was 86 per cent; with the 8-day treatment the cure rate dropped to 50 per cent. While our results are not strictly comparable with those of the above investigators, it does appear that the more intensive treatment regimes with lower total dosage by weight, as used by us in Schedules C and D, gave results as good or better than those reported by Halawani and Dawood (*loc. cit.*), especially if in our experiments we were to consider as cured, in addition to patients with negative urines, those patients passing only dead eggs in their urine at the end of the six months follow-up period.

SUMMARY

(1) A total of 207 adult males, infected with *Schistosoma haematobium* but living in a schistosome-free area, were treated with intramuscular injections of stibophen; 114 of these could be studied post-treatment for six months.

(2) Five different treatment schedules were tested with total drug dosages ranging from 21 cc. to 40 cc., and administered over periods of three to twenty days.

(3) Untoward reactions included anorexia, nausea, vomiting and a skin rash. Some patients also complained of weakness and generalized body pain. The severity and frequency of untoward reactions was positively correlated with the size and frequency of the individual doses, but not necessarily with total drug dosage.

(4) Cure rates, based on the clearance of all schistosome eggs from the urine by the sixth month post-treatment, ranged from 50 to 79 per cent. With adequate total dosage, the more intensive treatment regimes gave the higher cure rates.

(5) Concentrated treatment was followed by earlier relapse as shown by the re-appearance of living eggs, but degenerate eggs may also indicate unsuccessful treatment after an extended course with low dosages.

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RECTO SIGMOID POLYPS IN SCHISTOSOMIASIS

I. General Clinical and Pathological Considerations[1]

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The common intestinal changes associated with schistosomiasis were recognized soon after the discovery of the parasite. Laning from China in 1914 published a description of the pathological effects in the intestines. Madden (1922) reported the value of sigmoidoscopy for the diagnosis of papillomatosis in chronic Egyptian bilharziasis. Dew in 1923 stated that soon after the earliest symptoms of dysentery occurred in Egyptians with schistosomiasis, polypoid lesions appeared in the rectum and hemorrhages followed. Faust and Meleney (1924) in China established the pathogenesis of *S. japonicum* experimentally in dogs and pointed out the probable relation of epithelial proliferation and repair to the development of intestinal papillomata. Lambert and Burke (1928) showed that the presence of schistosomal eggs in the intestinal wall produced an inflammatory reaction with giant endothelial cells, mononuclear and eosinophilic cell infiltration and fibroblastic proliferation. Hutchison (1928) working in the Nile Delta of Egypt, found polyps associated with schistosomiasis of the large intestine; most common in the descending colon, the sigmoid colon and the rectum. He stated that symptoms and signs were variable and obstruction of the bowel frequently occurred in patients having numerous large polyps.

Koppisch (1943) noted that schistosomal infections in Puerto Rico were milder than those in Egypt. Gelfand reporting from South Central Africa listed no intestinal symptoms in 46 of 63 cases of intestinal schistosomiasis, and further stated that polyps were not found in 50 persons sigmoidoscoped. Gelfand (1950) reported a total of 185 autopsies performed on cases of schistosomiasis in Central Africa with no case of papillomata of the colon. He concluded that schistosomiasis in Central Africa differed from the Egyptian form commonly associated with "papillomata" reported by Sorour (1928) and Girges (1934). Girges designated papillomatous lesions as characteristic of the third stage of intestinal bilharziasis due to *S. mansoni*. He reported 32 per cent of his cases in the papillomatous stage with symptoms.

In light of previous findings that the polypoid lesions of the rectosigmoid colon are common and produce symptoms frequently in Egypt, a study of the pathology of these lesions was initiated in the Department of Pathology at the U. S. Naval Medical Research Unit No. 3, Cairo, Egypt.

MATERIAL AND METHODS

Patients having symptoms or signs of possible rectal or colonic origin at the Hurpur Memorial Hospital, Old Cairo, Egypt, American Mission Hospital,

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Tanta, Egypt, and U. S. Naval Medical Research Unit No. 3, Cairo, Egypt were subjected to rectosigmoidoscopy. When polypoid lesions were found, biopsy or surgical excision was used to secure tissues for study. Clinical laboratory examinations were performed to determine the presence of infection. Further study was made to determine the type of schistosome by urine sedimentation and rectal mucosa biopsy.

An unselected series of 155 autopsies on persons over 5 years of age were collected from Kasr el Aini Hospital and Abbassia Fever Hospital, Cairo and studied by gross and microscopic means to determine the presence of polypoid lesions of the large intestine and schistosomiasis. An additional series of 319 surgical cases of schistosomiasis from hospitals in Cairo and Tanta, Egypt, where tissues were available, were studied for bowel involvement.

The tissues used in this study were preserved in neutral 10 per cent formalin saline solution. Sectioned tissues were stained with hematoxylin eosin, and by Mason's trichrome, and Gridley's reticulum methods.

RESULTS

A total of 159 polypoid lesions were recovered by surgery or at necropsy and have been subjected to complete study.

FIG. 1. Photograph of barium enema of patient with polypoid lesions of the colon. Note that polyps appear in the sigmoid colon. Histological studies of the polyps revealed *S. mansoni* and *S. haematobium*.

Clinical Features. Arafa (1952) stated that the intestinal stage of the disease usually appears in 6–14 weeks after infestation, and the bilharzial papillomata of the colon appear as the intestinal disease progresses.

In the cases contained in this report the date of initial infection could not be elicited since all patients were Egyptian males from rural areas and had worked or bathed in the cercaria infested canals and irrigation ditches since early childhood. The age of the patients ranged from 19 to 40 years, with an average age of 29 years. Recent surveys in Egypt (WHO—1954) have indicated that the percentage of human infection rises rapidly from 14 to 40 per cent at the 5 year age level. On this basis, the duration of disease in the average case of the current series probably was greater than 14 years.

All patients had complaints referable to the rectum or colon. The symptoms ranged from tenesmus, cramping and small amounts of mucus to extensive bleeding, painful defecation and prolapse. The most consistent symptom was painful bleeding from the rectum. The duration of rectal bleeding ranged from 14 days to five years with an average of 3 months.

Other than for prolapse and occasional strangulation of a polyp external to the anal sphincter, evidence of polyposis necessitated proctosigmoidoscopic examination. Digital examination of the rectum was of no value in the diagnosis of schistosomal polyps of the colon. In addition to rectosigmoidoscopic examination, contrast medium-roentgenographic studies and direct visual examination were used to determine sites of polypoid formation and the extent of involvement.

Site of involvement was as follows:

	Per cent of cases
Rectal involvement	60.6
Sigmoid colon involvement	12.1
Combined rectosigmoid involvement	27.2

One patient showed polyps in the descending colon and one in the appendix.

Of the current series, ninety one per cent of the cases exhibited multiple polyps of the rectosigmoid colon.

Gross Pathology. The polyps varied considerably in size from 2 mm. to 20 mm. in diameter and from 2 mm. to 24 mm. in length. The average polyp was 8 mm. in diameter prior to fixation in formalin. The color was scarlet unless superficial ulceration was present to a marked extent. In that case, the ulcerated area was dusky to blackish gray in color. The color change can be attributed to superficial hemorrhage or clotted blood resulting from chronic bleeding.

The small polyps were sessile and as the polyps increased in size a pedicle could be seen. The largest polyps were usually pedunculated. Mucosa could be defined along the stalk. When a large sessile polyp was found, two or more foci of formation involving adjacent sites of bowel wall were present. Ulceration was noted more consistently in the rectal polyps in the sigmoid colon. Small polypoid projections from the polyps proper were frequently present causing a mulberry-like topography.

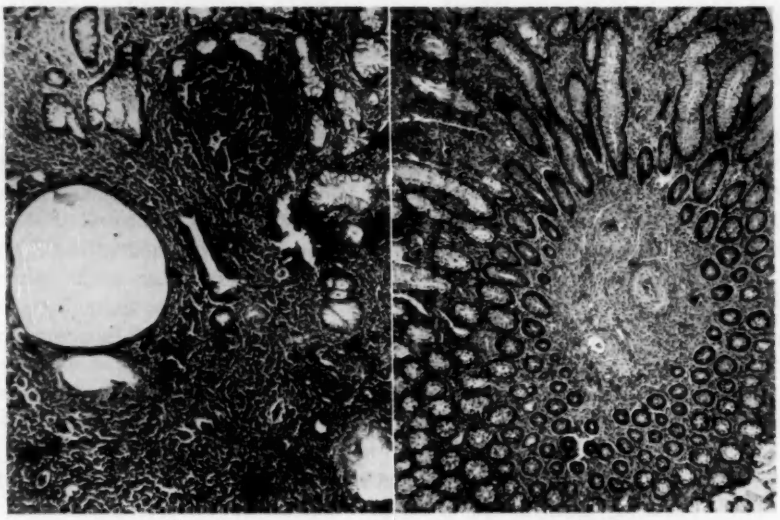

FIG. 2 FIG. 3

FIG. 2. Cross section of rectal polyp showing glandular disorganization. Note the granulomatous tissue about the calcified schistosomal eggs. × 85

FIG. 3. Cross section of polyp of the rectum showing the central fibrous core. Minute particles of schistosomal eggs are associated with the fibrous tissue and inflammatory cells. × 85

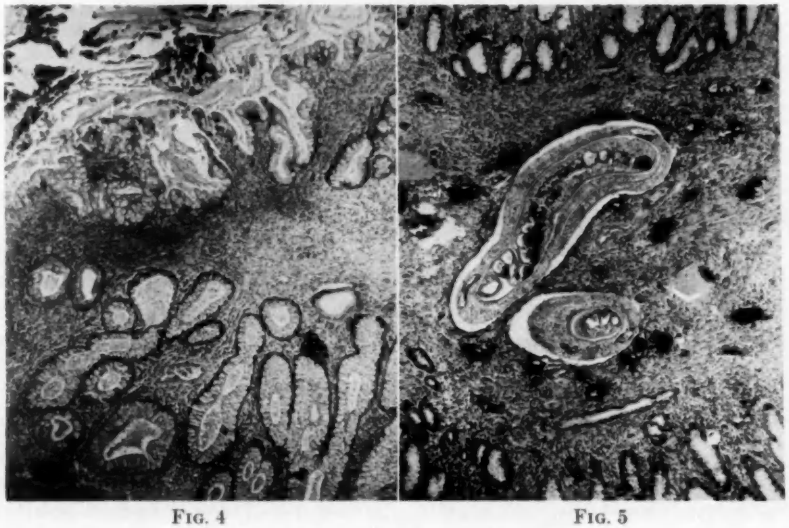

FIG. 4 FIG. 5

FIG. 4. Cross section of polyp showing adenomatoid hyperplasia and calcified schistosomal eggs near glandular structures. Mucoid material is abundant. × 85

FIG. 5. Cross section of a sigmoid polyp with schistosoma worm in the fibrous core. Calcified eggs are numerous. × 85

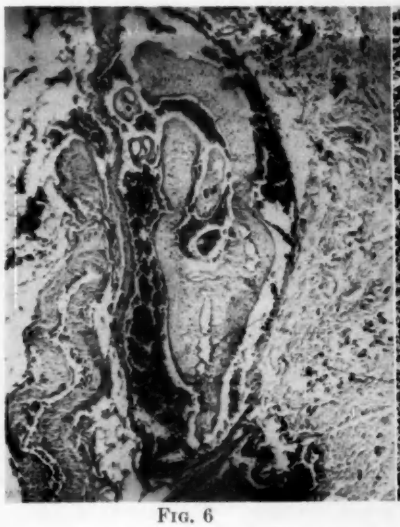

FIG. 6

FIG. 7

FIG. 6. Section of rectal wall adjacent to polyp. Dilated submucosal veins contain paired schistosoma worms. × 30

FIG. 7. Myxoid area adjacent to a viable egg of *S. mansoni*. Mononuclear and eosinophilic leukocytes surround eggs. × 250

FIG. 8

FIG. 9

FIG. 8. Schistosomal eggs migrating through the muscularis mucosa. Inflammatory cells are present in submucosa and mucosa. × 250

FIG. 9. Areas of fibrous tissue deposition in polyp associated with schistosomiasis. Note mucoid activity in mucosal cells. × 250

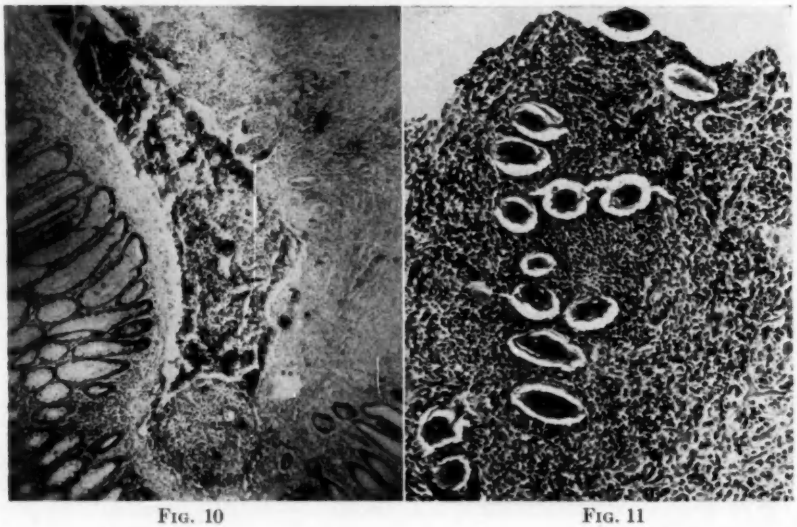

FIG. 10 FIG. 11

FIG. 10. Granulomas projecting into dilated vascular channels of polyp. The schistosomal eggs are viable and nonviable. × 98

FIG. 11. Egg mass in polyp associated with schistosomiasis beneath ulcerated margin. A distorted gland is seen at upper right corner. × 87

Upon section, the majority were friable and gritty. The mucosal layer frequently fell from the fibrous core. The color of the stalk ranged from white to pale gray. The gritty character was limited to the core except when adjacent bowel wall was attached to the polyp and the latter contained numerous calcified eggs. No specimens required decalcification and little technical difficulty was encountered after embedding.

Microscopic Pathology. The typical polyp associated with schistosomiasis was composed of a stalk of fibrous connective tissue projecting from the submucosa into the lumen of the bowel and partially covered with mucosa. The mucosa consisted of distorted glands showing varied degrees of mucoid activity, mucinous degeneration and adenomatoid hyperplasia. The mucosa was frequently interrupted by focal areas of ulceration. These were attributed to normal egg exit sites. Some showed large areas of ulceration with the mucosa replaced by granulation tissue. The mucosa remaining a part of the polyp was usually infiltrated by mononuclear cells, eosinophils, and a few polymorphonuclear leukocytes. The supporting tissue was composed of a mixture of fibrous connective tissue and muscle. The smooth muscle components derived from the muscularis mucosa could frequently be detected only by special stains. The density of the fibrous connective tissue varied with the age of the polyps. Inflammatory cell infiltration was of the same type as that seen in the mucosa but usually more intense with focal abscesses and numerous eosinophils. The younger lesions exhibited large numbers of blood vessels which diminished as fibrosis progressed.

Eggs were present in all polyps, usually in great number and the schistosome type could be identified. The eggs present within the supporting tissue as well as the mucosa varied from viable to nonviable. The viable forms were seen either in positions of migration in the tissues or were partially encased in young fibrous tissue. Very little cellular reaction was seen in the immediate vicinity of the viable eggs in transit.

The nonviable eggs varied from the early stages of disintegration of the contained miracidium to amorphous basic staining masses to small portions of chitin and other egg fragments. The early stages of disintegration showed intense cellular reaction with the formation of granuloma often referred to in the Egyptian literature as "bilharzioma" about the eggs. The eggs showing only small particles of chitin or shell components were usually the central focus of foreign body reaction with foreign body giant cells in the immediate area. The amorphous forms showed little cellular reaction about them though usually contained in a dense mass of fibrous connective tissue and contained calcium in varying degrees.

Polyps of long duration and those polyps from patients treated with antimony preparations revealed the greatest degree of fibrosis. The major portion of the structure was dense fibrous tissue without viable eggs. The disintegration, amorphous and calcified forms were frequent and caused difficulty in establishing the specific type of schistosome infection present. Inflammatory reaction in these polyps was minimal or lacking. The adenomatoid hyperplasia was not usually present and very little ulceration was seen.

Types and Forms of Schistosome Present. In this study a total of 159 polyps were examined to ascertain the specific type of schistosome present. The forms present in the polyps were used as the means of identification. It was found that by serial sectioning of the polyps the identity of the type could be established

TABLE 1

	Histological Tissue Method			Rectal Scraping Biopsy Method
	Number of polyps	Per cent of polyps	Per cent of cases	Per cent of cases
1. *Schistosoma haematobium* eggs only present	20	12.6	21.8	36.4
2. *Schistosoma mansoni* eggs only present	91	57.2	39.1	54.5
3. *Schistosoma haematobium* and *mansoni* types present	28	17.6	26.0	0.1
4. Schistosome type could not be established	17	10.7	13.0	0
5. Schistosome worms present	3	1.9	9.0*	0
6. Schistosome types other than *haematobium* and *mansoni*	0	0	0	0
Total	159	100.0		100.0

* In addition to percentage of 1 and 2.

and in turn allowed a more complete search for mixed infections. Rectal biopsies and scraping methods of identification agreed with the results of the fixed tissue method. In cases with few eggs present in the polyps and these mostly amorphous—calcified eggs were seen, the scrapings were of little additional value.

Table 1 shows the results of this study:

Incidence of rectosigmoid polyps

5,000 serial cases hospitalized at the American Mission Hospital, Tanta, Egypt between 1 January 1952 to March 15, 1953 (case records from 47314 to 52314) were analyzed. These consisted of a very high percentage of urban and rural Egyptians. Of these cases 213 were diagnosed primarily or secondarily as schistosomiasis. Forty (18.77%) of the cases had polypoid lesions of the colon that required operation for relief of symptoms. In 1953 a total of 378 surgical specimens were studied from the same institution and 14 cases (3.7%) were found to be polypoid lesions of the colon associated with schistosomiasis.

Of the 319 surgical specimens studied from the local Egyptian hospitals, 206 cases (64.5%) showed schistosomiasis of the bowel and of these, 42 cases (20.4%) had polypoid lesions of the rectosigmoid colon associated with schistosomiasis.

Of the 155 autopsies of persons over 5 years of age studied at NAMRU-3, 41 cases (26.5%) were found to possess schistosomal infections either active or quiescent. Of these cases of schistosomiasis, 7 cases (17.2%) possessed either single or multiple polypoid lesions of the rectosigmoid colon due to or associated with schistosomal parasitic forms.

In a related study in progress at this Unit, Dimmette and Ragheb (1954) examining a group of 100 cases of schistosomiasis with hepatosplenomegaly, 63 have been subjected to sigmoidoscopy, and of these 11 cases (17.4%) have shown polypoid lesions of the rectum or sigmoid colon attributed to schistosomal parasitic forms.

DISCUSSION

The polyps of the colon associated with schistosomiasis are best considered as complications of the disease process. In most cases of intestinal schistosomiasis the polypoid lesions are not present or do not reach a stage of development sufficient to be seen by gross inspection. In these studies the incidence rates of polyposis range from 17.2 to 20.4 per cent of the cases of schistosomiasis. In Egypt where both the *mansoni* and *haematobium* types are present approximately 45 per cent of our material was infected. This figure was derived by the combination of clinical examination and histological examination of surgical and autopsy material at this Unit.

The general incidence of polyps from all causes from this study was 9.2 per cent. This figure, however, cannot be accepted as the true incidence in Egypt inasmuch as no extensive surveys for polypoid lesions of the colon have been published. Mata (1944) reported a low figure of 0.9 per cent for polyposis in Argentina and Hanley and Hines (1954) a high figure of 9.5 per cent polyposis in Cuba. The Cuban survey reported 80 per cent of the polyps adenomatous

and made no reference to parasitic forms. With the WHO recent surveys in Egypt showing schistosomiasis up to 40 per cent human infection, and the current study and Girges reports indicating that 17 to 32 per cent of the schistosomal infections have polypoid lesions, the true incidence from all causes must be high. Further investigation and clinical studies must be made before a representative estimate can be reached.

From reports by authors (Koppisch 1943, Gelfand 1950) in other geographical regions of endemic schistosomiasis a low percentage of polypoid lesions of the colon is seen. The cause of the high incidence in Egypt is not fully known but speculation as to cause covers a rather wide range. In considering the high incidence in Egypt, factors of race, density of infections by parasites and infections of the gastrointestinal tract by bacterial agents are probably important. It is common knowledge that enteritis and colitis are frequent diseases in the Egyptian. Enteritis and colitis accounted for over 20 per cent of deaths in Alexandria and Cairo in a 3 month period of 1953 not considered as the diarrhea season. Higgins (1954) studying a group of 300 Egyptian village children for 6 months, isolated *Shigella* organisms from 80 per cent at least once. He also noted intestinal pathogens and parasites widely prevalent in these children. Jones (1954) working with a group of Egyptians found over 50 per cent of them harboring *E. histolytica*. The high rate of nonspecific clinical intestinal infection by bacteria and the constant infections by parasites may have some bearing on the cause of the high incidence in this locality. Such factors as race and status of nutrition have not been explored but possibly may be factors of importance. Koppisch (1943) implied that habits and socioeconomic factors unique to Egypt and Puerto Rico might cause the difference in the incidence.

This study indicates that the intestinal infection by schistosomes probably has been present for many years prior to the onset of symptoms and signs of polyp formation. The age of the patients in this study contribute to this supposition. All cases in this study had complaints referable to the rectum or colon. Although the intervals between onset of symptoms and examinations were for the most a period of months, the early stages of polyp formation are symptomless. The appearance of rectal bleeding brought most patients to institutions for diagnosis and treatment.

The polyp formations found in the rectosigmoid colon correspond to the region of the bowel with the greatest density of egg deposition. The occasional polyp associated with schistosomiasis found elsewhere in the colon was rare in this series. Since an overwhelming percentage of the cases showed multiple polyps, the term "polyposis" would apply in general.

The gross pathological findings varied with the age of the lesions and site. The alterations due to luminal trauma were common. The histologic findings in this study closely parallel those described by Faust and Meleney for *S. japonicum* in the intestines. The microscopic findings indicated that diagnosis of the etiological agent and type was not difficult. Further studies of the pathogenesis of the lesions with special reference to the adenomatoid hyperplasia are in progress and will be published in the future.

The finding of only three polyps with worms present probably indicates that

migration into a polyp is rare. The very infrequent finding of a worm or worm particle in the feces implies that this form rarely enters the mucosa layer of the bowel. The identity of the worms as to type is difficult in histological preparations.

A high percentage of the polyps contained eggs of the *mansoni* type and agreed with the predominance of *mansoni* found by rectal scrapings and biopsy of rectal mucosa. The histological examination revealed a higher percentage of *mansoni* and *haematobium* (mixed infections) in the polyps than was found by the scrapings and biopsy methods. This finding had its effect by lowering the percentage attributed to pure *haematobium* infections by other means. One disadvantage encountered in the histological sections was that an occasional polyp contains only calcified or degenerating eggs and the type could not be established by a single polyp examination.

SUMMARY

Polyps of the rectosigmoid colon are common complications of intestinal schistosomiasis and found present in 17–20 per cent of our cases. Schistosomiasis was found present in 26.5 per cent of our series of autopsy cases of persons over 5 years of age.

Patients with polyp formation of the rectosigmoid colon associated with schistosomiasis, ranged from 19–40 years of age, and presented complaints referable to the rectum and colon. The most frequent symptoms in our series were rectal bleeding and tenesmus.

Clinical and autopsy studies indicated that the polyps are primarily limited to the rectosigmoid portion of the bowel.

The gross and microscopic findings have been described in detail.

The majority (74.8%) of the 159 polyps contained eggs of the *mansoni* type whereas 30.2 per cent contained the *S. haematobium* type.

The combination of *S. mansoni* and *S. haematobium* was present in 17.6 per cent of the polyps. Worms were found in less than 2 per cent of the polyps.

Studies in regard to pathogenesis, mucosal changes, and relationship to neoplasia are in progress and are to be reported in the future.

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THE VALUE OF ROUTINE RECTAL BIOPSY IN THE DIAGNOSIS OF SCHISTOSOMIASIS[1]

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In an area where schistosomiasis is highly endemic, such as Egypt, the parasite may be missed if only the stools and urine of a patient are examined. The value of rectal biopsy in making a positive diagnosis of schistosomiasis has been emphasized by Ottolina (1947) who simplified the method of examination of the biopsy specimen.

In a group of 215 Egyptian villagers, the Bilharzia Team of the British Medical Research Council (1952) found 129 patients or 60 per cent to be positive for *S. haematobium* by direct examination of the urine. When those who were negative by urine examination were submitted to rectal scraping, 71 patients or 82 per cent were found to be positive. This observation indicates the relative inaccuracy of the examination of urine alone in surveying a population for schistosomiasis; one might expect analogous results in evaluating an individual patient. Methods of diagnosis of bilharziasis have been critically discussed recently by the Expert Committee on Bilharziasis of the World Health Organization 1953.

In the present study, rectal biopsy was done on 100 consecutive admissions to NAMRU-3. A variable number of stool and urine examinations were done on each patient. The purpose of the report is to compare the efficiency of rectal biopsy with serial urine and stool examinations.

METHODS AND MATERIALS

Patients were Egyptian males, usually villagers, admitted for a variety of illnesses—other than schistosomiasis—including brucellosis, amebiasis, typhus and nutritional deficiencies. They ranged in age from 17 to 50 years.

The individual's hospitalization was a matter of several weeks to 2 or 3 months. During that time, several stool samples were obtained. They were examined for eggs and parasites by the MIF or MIFC methods [Sapero and Lawless 1951, Blagg in press]. The personnel doing the stool examinations had wide experience in recognizing schistosome eggs.

Urine samples were examined in the usual way for microscopic findings at frequent intervals. In addition, each patient had one or more 24-hour urine specimen collected. The sediment of this specimen was centrifuged and examined for bilharzial eggs[3].

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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[3] The following method was used in examining 24 hour specimen.

1. Collect sample under toulene.

Rectal biopsy was obtained during the patient's convalescence. The patients were prepared by warm tap water enemas until clear, and then sigmoidoscoped in the knee chest position. When the sigmoidoscope had been withdrawn to the level of the middle valve of Houston, a small piece of mucosa was removed with biopsy forceps. Often there was slight bleeding at the biopsy site, but no serious difficulties were encountered. The time required for each biopsy was less than 5 minutes. Patient cooperation was excellent. No patient refused sigmoidoscopy, even when it was necessary to repeat the procedure one or more times.

The specimen was put in a tube of tap water and after a few minutes, removed and compressed between two glass slides. This method, described by Ottolina (1947), is simple and rapid and allows the differentiation between living and dead eggs, an important consideration if one is assessing the result of treatment. The living egg appears normal in shape, golden yellow in color, and contains an easily recognizable miracidium which will erupt from the shell if the tissue is allowed to stand on the microscope stage for an hour. The dead eggs are nearly black in color and the details of structure of the miracidium are distorted.

RESULTS

After one hundred cases had been submitted to routine biopsy, the results of biopsy were analyzed and compared with those of urine and stool examination with reference to the frequency with which eggs of schistosomiasis were discovered. The one hundred cases were consecutive except that cases with rectal pathology were omitted. Tables 1, 2 and 3 summarize the results.

From Table 1, one can see that a total of 61 positive biopsies were obtained from the 100 subjects sigmoidoscoped. Of these 61 patients, 60 had *S. haematobium* in the rectal biopsy and only 9 had *S. mansoni*, in eight instances both *S. haematobium* and *mansoni* were present. This finding emphasizes the point that the eggs of *S. haematobium* are very commonly located in the mucosa of the rectum as well as the bladder.

Routine urinalysis and stool examination—done in large numbers—infrequently revealed the eggs of schistosomiasis. Twenty-four hour urine examinations—done especially to discover schistosome eggs—were positive in 20 per cent of all urines so examined. Thirty-three of the 100 cases could have been diagnosed with this method alone.

To compare more fully the relative accuracy of rectal biopsy and 24 hour urine specimen examination, Table 3 is presented. In 32 patients in whom the 24 hour urine was negative, the rectal biopsy was positive. In the converse situa-

2. Put in a 2 L. cylinder and allow to stand for 4 hours.
3. Take 100 ml. of the sediment; let stand in a 100 ml. cylinder for one hour.
4. Take 15 ml. of the sediment in the second cylinder and centrifuge for 5 minutes at 1600 RPM.
5. Examine small amount of the spun sediment for presence of eggs by direct microscopy, without stain.
6. If the urine contains a heavy sediment of crystals, 2 or 3 drops of conc. HCl may be added to the sample on the slide without injuring the eggs.

TABLE 1

Results of rectal biopsy

	No. cases
Positive for	
S. haematobium	52
S. mansoni	1
Both	9
Negative	39
	100

TABLE 2

Results of urinalysis, stool examination and rectal biopsy—100 consecutive admissions

	No. of Spec. Examined	No. Positive Bilharzial eggs	Per cent Positive	No. of Patients
Routine urinalysis	864	28	3	14
Twenty-four hour urine exam.*	338	70	20	33
Routine stool exam. for eggs and parasites	1090	22	2.02	8
Rectal biopsy	100	61	61	61

* Specifically examined for bilharzial eggs.

TABLE 3

Comparison of rectal biopsy with 24 hour urine examination—100 cases

No. Times Rectal Biopsy Positive; 24 Hour Urine Negative	No. Times Rectal Biopsy Negative; 24 Hour Urine Positive	No. Times Both Negative	No. Times Both Positive
32	3	36	29

tion only 3 patients had negative rectal biopsy and positive 24 hour urine specimens.

DISCUSSION

From the results given above, it seems clear that if one has simple hospital or dispensary facilities—and a proctoscope—rectal biopsy is the method of choice in attempting to diagnose schistosomiasis in an individual patient or in attempting to estimate its frequency in a given population. True, the rectal biopsy requires a few minutes of the physician's time and is somewhat discomforting to the patient, but the considerable time saved the laboratory and the greater diagnostic value of the procedure justify these disadvantages. One might expect the examination of concentrated urine samples to be superior to examining mucosa from the rectum in revealing the eggs of *S. haematobium* in patients with this parasite. However, as indicated above, rectal biopsy was far more effective in detecting *S. haematobium* than multiple urine examinations. This is in accord

with the observation recently recorded by the Expert Committee on Bilharziasis that "... *S. haematobium* and related species can often be detected in rectal biopsy snips even when none of these eggs can be found by examination of a specimen of urine." (WHO 1953).

In our experience, only 3 cases of schistosomiasis—of a total of 64 diagnosed—would have been missed if the 24 hour urine examination, done an average of 3.4 times per patient, had not been performed. Thus, if the procedure of rectal biopsy is available, there is little to justify the examination of 24 hour urine specimens in attempting to diagnose schistosomiasis.

SUMMARY AND CONCLUSIONS

1. One hundred consecutive admissions, consisting of Egyptian males of the Cairo and Delta region, admitted usually for brucellosis or amebiasis, were subjected to rectal biopsy.

2. There were no complications to the procedure. Sixty-one cases were found to have schistosome eggs; sixty, *S. haematobium*; one, *S. mansoni*; and eight had both *S. haematobium* and *S. mansoni* present.

3. An average of 3.4 twenty-four hour urine examinations for schistosome eggs were done per patient. A total of 33 patients were found to have eggs by this method.

4. In 32 patients the rectal biopsy was positive when examination of 24-hour urine specimens had been negative. Conversely, in only 3 patients were eggs discovered in concentrated urine specimens in patients in whom a rectal biopsy had been negative.

5. Rectal biopsy is the method of choice in establishing the diagnosis of vesical schistosomiasis.

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THE EFFECT OF *SCHISTOSOMA MANSONI* INFECTIONS ON LIVER FUNCTION IN MICE

II. Further Studies on Intermediary Metabolism[1]

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In a previous report (Daugherty, Garson and Heyneman, 1954) it was shown that mouse liver minces, six weeks after infection with heavy dosages of *Schistosoma mansoni* cercariae, consumed oxygen in a Tyrode medium at a rate little different from that of normal tissues, despite the fact that other, more specific, liver functions were found to be altered. As a part of a series of studies seeking to determine the physiological effects of schistosomiasis on the host, the following study was initiated in the hope of (1) more fully describing the effects of this infection in terms of liver respiration and (2) establishing criteria that could be used to study the possible existence of a toxic elaboration from the parasite, its eggs, or the diseased liver tissue. A repetition of some of the earlier work was undertaken under modified conditions. Preliminary experiments along this line indicated that the succinoxidase system was affected in the infected livers. Assays of succinoxidase activity, cytochrome oxidase activity, and the activity of the succinic dehydrogenase system were, therefore, compared in preparations from infected and non-infected livers. In addition, total N_2 and collagen were determined in the infected and control animals in an effort to explain the findings obtained from these assays. Preliminary reports on the effect of the infection on other liver metabolic systems are also given.

GENERAL METHODS

The liver tissues used in these experiments were obtained from mature male albino mice bisexually infected by tail exposure to 150–160 *S. mansoni* carcariae, according to the method of Olivier and Stirewalt (1952). A continuous supply of mice was maintained by weekly infections. The mice were weighed and sacrificed during the sixth and seventh week following infection. The livers were removed and either immediately homogenized in cold glass distilled water in chilled glass-homogenizers or they were minced for surviving (intact) tissue studies. The mincing was done by repeatedly cutting the tissues after which they were thoroughly mashed on the bottom of an iced Petri dish with a chilled, stainless steel spatula. The tissues were kept cold at all times. This method of handling the surviving tissues was found to be superior to the use of slices in reproducibility of results and ease of handling, since it was desirable to place identical wet weights of tissue in the separate reaction vessels. The weighing was quickly

[1] This study was sponsored by the Army Medical Service Graduate School and was supported (in part) by the Medical Research and Development Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

and easily done by using tared weighing papers and a torsion balance. A special aluminum spatula was made to introduce the mince into the Warburg flasks.

In those experiments involving the use of homogenates, preliminary runs were made to determine favorable media for the various assays. The media used were found to give fairly constant readings over a period of 40–60 minutes. They were, in part, taken from those described for rat liver (Umbreit, *et al.*, 1949). The more specialized methods are described below in connection with the separate experiments.

RESULTS

A. *Surviving tissues.* In the previous studies on the oxygen consumption of liver minces from *S. mansoni* infected mice (Daugherty *et al.*, 1954), a phosphate-buffered Tyrode solution (Difco) was used as the incubation medium. In the present tests, phosphate-buffered Krebs-Ringer (Umbreit *et al.*, 1949) was substituted to measure endogenous respiration. As a means of estimating the ability of the tissues to respire on certain specific substrates, separate portions of the basic Krebs-Ringer medium were fortified by the addition of glucose, sodium pyruvate, and sodium succinate, to a final molarity of 0.05 M. These substances were chosen as they represent important and oft-studied intermediate substrates in the metabolism of carbohydrates. Three ml. of each medium were used in the tests and the reaction mixtures were completed by the addition to each of 300 mgm. of fresh infected or uninfected liver mince. The mixture was incubated in the Warburg apparatus at 38° C. in air. The results of these determinations are given in Table 1.

It may be noted that in all cases, except when sodium succinate was added to the basic medium, the respiration of the infected tissue differed little from that of the normal tissue. It frequently exceeded the uninfected tissue in activity. This is in agreement with earlier findings (Daugherty, *et al.*, 1954). Both tissues showed an ability to increase their oxygen consumption in the presence of glucose and pyruvate by small but nearly equivalent amounts. Although both tissues responded actively to the presence of succinate, the infected tissue failed to oxidize the succinate as rapidly as did the uninfected tissue.

In all of these studies it was noticed that larger variations existed between

TABLE 1

Effect of S. mansoni *infection on the respiration of mouse liver minces*

300 mgm. of fresh liver mince in each vessel. The results are given in microliters of oxygen per 10-minute period based on 3 equivalent readings. Numbers in parentheses are the experiments on separate mice run in duplicate.

Tissue	Medium			
	Krebs-Ringer	+.05M glucose	+.05M sodium pyruvate	+.05M sodium succinate
Uninfected liver (6)	28.6 ± 3.2	32 ± 4.0	39.6 ± 3.7	76 ± 4.1
Infected liver (6)	34 ± 6.4	37 ± 5.7	36.6 ± 8.1	57 ± 6.3

separate determinations on infected livers than between those on uninfected ones. This was probably the result of an inability to control the number of cercariae that established in the mice, although it is quite possible that this variation may be attributed, in part, to a variation in the innate response of the mouse host to the infection, *per se*. As a means of obtaining information on these points, the variation in total established worms was determined in twenty-five mice infected under conditions identical with those of the experiments above. With the 150 cercariae dose an average recovery of 69 ± 21 adult worms was obtained. These worms represented both males and females and included those found in pairs as well as those occurring singly. Both the portal and mesenteric areas were included in the count. In the present experiments only those livers showing grossly the manifestations of an acute infection were used.

B. *Homogenates.* In view of the results obtained following the addition of sodium succinate to the liver minces from infected and uninfected mice, it seemed desirable to make a more detailed study of the succinoxidase system in relation to schistosomiasis. The succinoxidase assays were carried out in a medium containing, in final concentration, .033 M phosphate buffer, pH 7.4; 3.2×10^{-5} M cytochrome c; .0004 M $CaCl_2$; .004 M $AlCl_3$; .05 M sodium succinate; 20 mgm. of fresh liver (5 per cent homogenate); and water to make 3.0 ml. 0.1 ml. of 2 N KOH was in the center well of the Warburg reaction vessel and the reaction was carried out in air at 38° C. Controls from which the succinate was omitted were run and the activity of the complete system corrected by these results. In only a few cases was there any discernible activity on the part of the controls. The results of the determinations on the succinoxidase activity are given in Table 2.

These data and others in the table were calculated in terms of oxygen consumption on a fresh tissue basis. It was felt that this gave the most direct physiological relationship in view of the fact that it was organ function upon which the major interest was focused. At the same time it seemed to be a safe basis upon which to compare the respiration of the infected and uninfected livers since no difference was found to exist in the endogenous respiration of the two tissues and, therefore, apparently no difference in the amounts of total active tissue. The increase in water content that was previously reported in infected livers (Daugherty, *et al.*, 1954) was apparently not sufficient to effect a sig-

TABLE 2

Effect of S. mansoni *infection on the succinoxidase activity of mouse liver*

20 mg. of fresh tissue (5 per cent homogenates) in the succinoxidase and succinic dehydrogenase determinations. 10 mg. fresh tissue (5 per cent homogenate) in the cytochrome oxidase determination. Results are given in microliters of oxygen consumed in 10 minutes based on three equivalent readings. Numbers in parentheses are the number of determinations on separate animals in duplicate.

Tissue hemogenate	Enzyme system		
	Succinoxidase	Cytochrome oxidase	Succinic dehydrogenase
Uninfected liver (6)	72 ± 4.3	76 ± 5.5	21 ± 2.6
Infected liver (6)	35 ± 7.1	49 ± 6.7	9 ± 2.3

TABLE 3

Total nitrogen and collagen in mouse liver infected with S. mansoni

Nitrogen figures are given in mgm. per gram of dry tissue. Collagen is given in per cent of tissue wet weight in duplicate determinations. Numbers in parentheses are the number of determinations in duplicate on separate animals.

Tissue	Total nitrogen	Collagen Animal #1		Collagen Animal #2	
Uninfected liver	109.8 ± .8 (12)	.42	.63	.39	.26
Infected liver	113.2 ± 1.8 (12)	1.20	1.04	.97	1.09

nificant change in endogenous oxygen consumption. It is not contended that all of the tissues involved in the assays were hepatic tissues. This, however, is not a critical point since an elucidation of the mechanism whereby the succinoxidase activity of the preparation was depressed, regardless of tissue involved, was considered an important step toward determining whether toxic principles are involved in schistosomiasis. As a means of further checking on the amount of active tissue, total nitrogen determinations were made on the livers by colorimetric (Umbreit *et al.*, 1949) and Kjeldahl procedures. These results also indicated no reduction in the enzymatically active components of the tissues (Table 3) assuming that total nitrogen is a true index of total protein (enzyme) present (Umbreit *et al.*, 1949). It will be noted, in fact, from Table 3 that the total nitrogen content of the infected liver was somewhat higher than that of normal liver. In spite of this, a marked difference existed in the abilities of infected and uninfected liver homogenates to oxidize sodium succinate under the conditions of the experiment. These findings agree closely with the results of the liver mince experiments on the sodium succinate substrate. Since the succinoxidase system, as measured by the technique described above, includes several enzyme reactions, an effort was made to study some of these separate systems. Determination of cytochrome oxidase activity was carried out in the following medium: .033 M phosphate buffer, pH 7.4; 3.2×10^{-5} M cytochrome c; .0004 M $AlCl_3$; .0114 M sodium ascorbate; 10 mgm. fresh liver (5 per cent homogenate), and water to make 3.0 ml. Suitable controls were included. For the estimation of the effect of the infection on succinic dehydrogenase activity the medium used for the succinoxidase assays was altered by the substitution of 1.3×10^{-4} M methylene blue for the cytochrome c in order to bypass the cytochrome reductase and cytochrome oxidase systems. It was not intended that this represent an optimum condition for this determination. The results of these studies are found in Table 2. It is readily seen that the infected mouse liver homogenates were unable to equal the ability of the normal liver to oxidize either sodium succinate or reduced cytochrome c. The low figures of oxygen consumption in the methylene blue experiments were not unexpected since there are few vertebrate tissues in which this dye adequately substitutes for the cytochrome system in the electron transfer mechanism.

C. *Effect of infected liver extracts and adult worm extracts on the succinoxidase*

activity of normal liver homogenates. An attempt was made to extract infected liver tissues (6–7 weeks following exposure to 150 cercariae) and adult *S. mansoni* worms for the possible presence of a material toxic to the succinoxidase system. Crude extracts were prepared as follows: a heavily infected mouse liver (3.4 gms) was forced through a sieve with 0.295 mm openings to remove the connective tissue framework. One gram of this mince was then suspended in 10 ml. of a 0.88 M sucrose solution and centrifuged at 24,000 × g for 30 minutes to remove the mitochrondria. This was necessary in order to remove the succinoxidase activity of the extract since this enzyme system is known to be associated with the mitochrondrial fraction of the cell. The supernatent was then added to normal liver succinoxidase assay mixtures in amounts varying from 0.3 to 0.6 ml. Controls were run omitting the normal liver homogenates. No succinoxidase activity could be measured in the infected liver extract alone and no effect of the addition of infected liver extract could be obtained on the activity of normal liver homogenates. Worm extracts prepared from 28.1 mgm. of lyophilized worm (150–200 worms) similarly treated, also gave negative results.

D. *Determinations of collagen in infected mouse liver.* Meleney *et al.*, (1953), described the fibrosis and scar formation that resulted from local egg and worm lesions in mouse livers following bisexual infection with *S. mansoni.* In the present series of experiments, the fibrous nature of infected mouse liver frequently caused some difficulty in obtaining reliable homogenates of this tissue. Upon correlation of these findings with the increased total nitrogen in infected mouse liver, it seemed desirable to determine the extent of the invasion of white fibrous connective tissue into the infected liver and the part it might play in the reduction of succinoxidase activity and possibly other more specific liver functions. Accordingly, studies were made on the collagen content of normal and infected mouse livers. Two mice, infected as those above, were sacrificed and the livers analyzed for collagen in duplicate determinations by the method of Lowry, Gilligan, and Katersky (1941). Two control mice were also run. The results, given in Table 3, demonstrate that a marked increase in liver collagen accompanied the present *S. mansoni* infections. The wide variation in results is similar to that shown by Lowry *et al.*, (1941), for rat liver.

E. *Studies on the activity of other enzyme systems in infected mouse liver.* These assays on other systems were made to study the possibility that the depressed succinoxidase activity of infected liver preparations was the result of a specific inhibition. The systems chosen apparently bear no relationship to succinoxidase activity, *per se*, but they do provide information on other aspects of the overall problem of liver function.

One of the most striking manifestations of a grossly infected mouse liver is the abundant deposition of a dark pigmentation. This pigmentation is now being studied to determine its origin and possible role in the hepatic effects of schistosomiasis. During the early stages of this study, attention was briefly turned to the investigation of melanin, during which assays of tyrosinase activity were made. This assay was carried out in the following medium using catechol as the substrate: 1 ml. of 0.5 M potassium phosphate buffer, pH 7.4; 0.2 ml. of 0.1 M

catechol; 0.5 ml. of 10 per cent liver homogenate and water to make a total volume of 2.0 ml. The incubation was carried out in the Warburg at 38°C. Under these conditions there was no detectable difference in the tyrosinase activity of normal and infected liver homogenates.

Several workers have reported abnormal blood sugar levels in human beings infected with schistosomiasis (Day, 1924; Erfan and Camb, 1933; Seife and Lisa, 1950). This would seem to indicate a potential involvement of hepatic function, as well as pancreatic function. Accordingly, several assays on the activity of the glycolytic mechanism were carried out on normal and infected liver homogenates using the method described by LaPage (1948). The results of these assays have not been entirely completed and they will form a part of a future report. However, it is pertinent at this time to report that although a reduced activity (*ca.* 15–20 per cent) of the glycolytic mechanism was found in the infected liver homogenates, it did not approach, in severity, the effect noted above on the succinoxidase system.

DISCUSSION

From the results given in the foregoing section it is apparent that an infection with *S. mansoni* is accompanied by an interference with the functioning of certain metabolic enzyme systems in mouse liver. It is impractical at the present time, to speculate on the relationship of the present results on liver homogenates and minces to the grosser and, perhaps, more specific functions of the host liver. However, these results are of considerable consequence in that they provide criteria for an evaluation of the several possible explanations as to the mechanism of some of the effects of schistosomiasis on the host animal. During the course of the present study two such possibilities have been recognized. The first of these is based on an explanation of the effects of the infection in terms of mechanical disruption of liver organization and dilution of active hepatic tissues with tissues and other substances having lesser activity, particularly where specific liver functions are concerned. According to this explanation, the results of the experiments on the liver homogenates, which are described here, must be considered artifacts resulting from the inclusion in the tissue sample of materials having a lower level of activity than the true liver constituents. An analysis of the results given in the preceding section do not seem to bear this out in full. If there was sufficient extra-hepatic tissue in the infected liver to account for the abnormal function of the succinoxidase system, it would seem that other systems would have also been affected to a comparable extent. This was not the case. The over-all oxygen consumption of the liver minces on a non-nutrient medium and on glucose and pyruvate fortified media did not show the expected reduction in activity in the infected liver nor did the livers differ to any significant extent in their nitrogen composition. In addition, preliminary results on the activity of the series of enzyme systems concerned with the degradation of fructose-1,6-di-phosphate, although showing a somewhat reduced activity in the homogenates from infected livers, did not vary to an extent comparable to the reduction in succinoxidase activity. There was no effect on

tyrosinase activity. Other systems are being studied and these investigations will be the subject of a future report. The only part of the present data that supports an interpretation of the present results on the basis of an invasion of non-living material is the increased collagen content of the infected liver. However, the collagen content of normal liver is relatively small and it seems doubtful that the increase following infection noted herein is sufficient, by itself, to account for the gross disturbances reported above.

The other basic hypothesis given in explanation of the effects of schistosomiasis on liver function assumes the intervention of toxic substances elaborated from the worms, their eggs, or the necrotic or metabolically abnormal liver tissues. Despite the fact that, by the simple experiment described in the preceding section, no toxic substance could be found, the possibility that such a substance exists should not be ruled out. It is not unreasonable to suggest that this failure to demonstrate a metabolic poison may have resulted from the fact that (1) the toxin must act or accumulate over an extended period of time in order to be effective or (2) perhaps the toxin was precipitated with the mitrochondrial fraction. Further work on these points is needed, and much of it is in progress. For example, in more recent studies on the activity of metabolic systems associated with the mitochondrial fraction of the mouse liver it was noted that nearly all of the intense pigmentation that may be seen in the liver of heavily infected animals came down with the mitochondria during centrifugation (unpublished). This may be of importance in interpreting the effect of the infection on succinoxidase activity, since this system is usually associated with the mitochondria. Investigations into the possible role of this pigment in the manifestation of schistosomiasis as it relates to liver function are at present being actively pursued. Through these experiments it is hoped that more specific information may be obtained on the nature of the succinoxidase deficiency. It is also anticipated that these studies taken as a whole will finally either confirm or reject the existence of a toxic substance associated with schistosomiasis in mice.

SUMMARY

The livers of mice infected with *S. mansoni* showed a reduced ability to oxidize succinic acid despite the fact that endogenous respiration was little affected. The effect on the succinoxidase activity is possibly, in part, attributable to the incorporation within the liver of extra-hepatic tissues, but the existence of a toxic influence by the infection was not ignored. The total nitrogen of infected liver was slightly higher than that of normal liver and an increase in liver collagen accompanied the present infections. No effect of preliminary extracts of infected liver and adult worm were obtained on the succinoxidase system of normal mouse liver. Tyrosinase activity of mouse liver was unaffected by the infection but the glycolytic mechanism was somewhat reduced in activity.

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STUDIES ON THE TREATMENT OF OPISTHORCHIS VIVERRINI IN HUMAN INFECTIONS WITH QUINACRINE HYDROCHLORIDE AND CHLOROQUINE PHOSPHATE[1]

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Many therapeutic agents have been tested in experimental animals and in man for the treatment of liver fluke infections and some encouraging results have been reported. However, there is, as yet, no single treatment that has been widely accepted. In the treatment of *Clonorchis sinensis* and *Opisthorchis felineus* infections, the long list of drugs tested are, at best, of limited efficacy and not without danger. To the authors' knowledge, no drug has ever been suggested in the literature for the treatment of *Opisthorchis viverrini* infections.

In view of the fact that opisthorchiasis, due to *O. viverrini*, represents a major clinical and public health problem in Thailand (Sadun, 1953, 1953a, 1954, 1954a) some attempts have been made to find a therapeutic agent which would be helpful in the treatment of this infection. Some of the results obtained have been reported in a preliminary form (Sadun and Chamnarnkit, 1953).

The only two drugs readily available to us in Thailand which possessed the desirable characteristics for therapy in liver fluke infection were quinacrine hydrochloride and chloroquine phosphate. Quinacrine is rapidly absorbed after oral administration and can be found evenly distributed in the body tissues a few minutes afterwards. Two and one-half hours after administration, the concentration appears to be relatively greater in the liver, gall bladder and intestines. It is believed that the drug is absorbed by the duodenum and carried to the liver where it is excreted with the bile back into the duodenum to pass once more into the liver with the portal blood. It is a relatively nontoxic drug. Chloroquine is available in tablets of 0.25 gm., equivalent to 0.15 gm. of the base. It is administered by mouth and has a bitter taste to which children often object.

Chloroquine is absorbed from the gastrointestinal tract somewhat more rapidly than quinacrine. There is considerable accumulation in various organs and tissues, the quantity stored being somewhat proportional to the dose ingested. These deposits occur in nucleated cells, particularly those of the liver, spleen, kidneys and lungs. These organs may have a concentration of the drug between 200 and 500 times that present in the plasma. On the other hand, the concentration in the central nervous system is usually no more than 10 to 25

[1] The authors wish to express their appreciation to Dr. Pramern Chandavimol, Chief of the Division of Communicable Disease Control, Thai Department of Health, for the constant and sympathetic interest in the project.

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times that of the plasma. Much of the chloroquine ingested appears to be metabolized in the body and only 10 to 20 per cent is excreted unchanged in the urine. Excretion is slow, although somewhat more rapid than that of quinacrine. There is very little difference between the toxicity of chloroquine and that of quinacrine. All available data indicate that chloroquine is not likely to produce any prolonged side effects and all unpleasant symptoms subside when the administration is suspended (Boyd, 1949).

In view of the ease of administration, ready availability, low toxicity, high concentration in the liver and/or gall bladder, slow excretion, and proven previous action as anthelminthics, quinacrine and chloroquine were tried in the treatment of infections with *O. viverrini*.

MATERIALS AND METHODS

Although nearly 150 persons infected with *O. viverrini* were selected for this study over a period of about 20 months, data at the end of treatment are available on only 65. The rest either failed to complete the course of treatment or failed to submit stools at regular intervals, as instructed. All of the patients were Thai, living in the Northeast region or recently moved from the Northeast to Bangkok. Some of the individuals received these drugs as out-patients. Whenever possible, however, the individuals selected for this study were hospitalized for the entire duration of treatment; thus, the chances of reinfection were minimized by having them under direct observation at all times. Most of the patients, after discharge, failed to return to the hospital for follow-up studies and could not be reached. In order to obviate this difficulty, arrangements were made with the Royal Thai Army to treat some of the infected soldiers. Follow-up studies could thus be carried out on practically all of the treated soldiers. Of the 65 persons included in this series, 8 were treated with quinacrine, and 57 with chloroquine. The doses employed in treating the first members of this series were somewhat empirical and were based on the doses of quinacrine and chloroquine recommended for other parasitic infections. In the later part of this study, persons were treated according to experience gained in the earlier experiments. In determining the doses, the age of the patients and the fact that Thai, on the average, weigh less than Europeans or Americans, have been taken into consideration. Dosages of chloroquine are expressed in terms of the base. All drugs were given in the presence of the authors.

Acid ether concentrations of stools were carried out on a few cases. However, since they could not be carried out regularly under field conditions, they were discontinued. Therefore, patients that have been reported as negative may still have passed a few eggs in the stools in amounts so small as not to be detectable by simple smear examinations.

The feces of each patient were examined quantitatively (Stoll and Hauscheer, 1926) at least two times before treatment in order to establish a reasonable constancy of egg elimination. This method can be utilized to measure the effect of the drug on the egg production of the worms and indirectly on any diminution in the number of worms that might occur during treatment or subsequently. It

should be pointed out, however, that a "reduction of egg count following administration of a therapeutic dose of a drug does not, in itself, indicate a parallel reduction in worms" unless the egg count drops suddenly and remains low over a considerable period of time (Faust *et al.*, 1927). Counts were repeated at regular intervals from the time the patient was given the first dose of the drug up to the last day of treatment. Patients were instructed to return to the hospital or to the dispensary as often as possible during the first 90 days from the termination of treatment. Altogether, follow-up studies were possible on 22 persons.

PRESENTATION OF DATA

1. *Treatment with quinacrine hydrochloride.* A series of 8 male patients is reported here. Six of them were school children, 13 years old, and two were soldiers, 21 years old. All infections were of moderate intensity. The number of eggs per gm. of formed feces varied from 1,200 to 6,400 with an average for the group of 4,070. Each patient was given 0.4 gm. of quinacrine per day for 3 days. This dose was followed by a smaller dose for periods of time that varied from 2 to 5 days. Altogether, the two adults received 2 gm. of the drug and the children received 1.6 or 1.7 gm. each. Sodium bicarbonate and phenobarbital were sometimes given together with quinacrine in order to minimize gastric discomfort.

At the end of treatment, 3 persons had a higher egg count, four had a lower, and one had the same as before treatment. The average eggs/gm. for the group, after treatment, was 4,100. Two patients returned for follow-up studies 30 days after termination of treatment. One of them had a higher and the other a lower eggs/gm. than at time of discharge. No consistent change in the egg count was observed as a sequel to the administration of the drug either during or after treatment.

2. *Treatment with chloroquine phosphate, series 1.* In this series, 25 patients, 8 males and 17 females, were selected. Except for a girl 6 years old, all the patients were adults ranging in age from 16 to 56 years. The average eggs/gm. for the group before treatment was 8,240. The intensity of the infections varied from very light to very heavy. Each adult patient was given 0.6 gm. a day of the base of chloroquine for 2 consecutive days followed by 0.3 gm. daily for the rest of the treatment. The child 6 years old received one half of these dosages. Of the 25 persons, 19 received treatment for 10 days, and 6 for 11 days.

At first, the most conspicuous effect of treatment was to bring about an apparent increase in egg production. The stimulative effect of the drug was most marked in Patient 9A where the eggs/gm. jumped abruptly from 3,000 to 14,800. This hyperproduction of eggs was not directly connected with the evacuation of immature eggs and was not observed in every patient. In fact, in some patients (22H and 45A) the eggs/gm. declined shortly after administration of the drug. Five to seven days after the beginning of the treatment, two patients (22H and 65A) were negative; 15 had a lower, and 8 had a higher egg count. The average for the group was 5,750 eggs/gm. At the end of treatment, 3 patients (18H, 22H, and 65A) were negative by smear examinations, 17 had a lower and 4 had a higher egg count. One patient had the same count as before treatment. The average for the group was 3,510 eggs/gm.

Series 2. Since in the previous series the egg counts of most patients decreased up to the last day of treatment, it was decided to try the effect of extending the period of treatment. Of the 9 patients selected, 4 were males and 5 were females. All of the patients were adults ranging in age from 16 to 35 years. The average eggs/gm. for the group before treatment was 4,710. On the whole, infections were much lighter than in the previous series and varied from 400 eggs/gm. (18B and 226) to 18,800 eggs/gm. (26B). Each patient was given 5.4 gm. of chloroquine base distributed as follows: 0.6 gm. daily for 2 days followed by 0.3 gm. daily for 14 days. As in the previous series, there was an apparent increase in the egg production shortly following the beginning of treatment. Three to four days after initiation of treatment, 4 out of 6 egg counts were higher than before and 2 were unchanged. Five to 7 days after treatment, five persons in the group had a lower egg count than before treatment. The average eggs/gm. for the group was 3,800. After 10 to 12 days of treatment, 3 patients (18B, 226, and 228) were negative for *Opisthorchis* eggs and all the others but one (543) had a lower egg count than before receiving chloroquine. The average eggs/gm. for the group was 1,710. This downward trend was continued up to the end of treatment when 5 out of 9 patients had become negative, and 4 had a lower eggs/gm. The average egg count for the group at end of treatment was 1,180 eggs/gm.

Series 3. On the basis of the previous results, it was decided to increase both the dosage and the length of the course of treatment on the next series of patients. Also, in order to determine whether the reduction in egg production following treatment is temporary or long lasting, an attempt was made to examine patients after treatment had been omitted for variable lengths of time. In order to achieve this end, the cooperation of members of the Royal Thai Army as well as of other institutionalized persons was requested. In this series, 23 adult males from 19 to 47 years old were selected. Infections of as wide a range as possible in intensity were chosen. Persons with as light an infection as 400 eggs/gm. (1179U) and as heavy as 30,800 (60U) were included in this study. The average egg count for the group before treatment was 7,570 eggs/gm. Two patients (CI and SI) were each given 6.9 gm. of chloroquine base over a period of 20 days and the others were each given 7.8 gm. of the base over a period of 23 days. As in both of the previous series, 3 to 4 days after the beginning of treatment, most people had a higher egg count than before treatment. The stimulative effect of the drug was most marked in patients 272S and 60U where the eggs/gm. increased abruptly from 1,600 and 30,800 to 4,000 and 72,400 respectively. Five to 7 days after the beginning of treatment, 11 patients had a lower, 4 had the same, and 8 had a higher egg count than before treatment. The average for the group was 5,700 eggs/gm. Ten to twelve days after treatment, 14 patients had a lower egg count and 9 had a higher one. A sudden decline in eggs/gm. was observed in patients 240U and CI. The average for the group was 4,670 eggs/gm. At the end of treatment, 10 patients were negative for *Opisthorchis* eggs, 11 had a lower and 2 (38S and 1388S) had a higher egg count than before treatment. The average for the group was 940 eggs/gm.

All the patients were instructed to return for follow-up studies. Only 3 failed to do so because they had moved to another region. Of the 20 available, 4 were

observed for 10 days after treatment was discontinued. One of them (38S) who had failed to respond to treatment had a higher egg count. Two (60U and SI) had 30,800 and 6,400 eggs/gm. before treatment, 2,400 and 2,000 eggs/gm. at the end of treatment, and 1,000 and 800 eggs/gm., respectively, 10 days later. A fourth patient (1179U) who had become negative as a result of treatment was still negative 10 days after treatment was discontinued. One patient (135IS) was observed for 24 days after chloroquine was discontinued. The egg count was still considerably lower than before treatment. Thirteen patients were observed for a period of time between 40 and 42 days after administration of the drug had been discontinued. Of these, 4 were still negative for *Opisthorchis* eggs and 2 (272S and 346S) had an egg count even higher than at the beginning of treatment. The other 7 were still passing only a few eggs with their stools. Two patients (66U and 97U) were followed for 90 days after treatment was discontinued. One of them (66U) was still free of the infection and the other had a lower egg count than at the end of treatment.

DISCUSSION

In studying the effect of therapy on infections with *O. viverrini*, one is aware of the difficulty of evaluating the results of treatment in human cases. In fact, such cases would have to be followed for a considerable time to be sure that the reduction of eggs in the stools following treatment really indicated that the parasites had been killed in the liver. This is not practical since the patients would have to be removed to areas where reinfection is not likely to occur. Furthermore, as indicated in the introduction, many drugs that gave promising results against liver flukes in experimental animals failed to be effective against flukes in humans even when the same species of parasite was involved. It is with these considerations in mind that the results of the above experiments will be analyzed.

The results of the studies on quinacrine hydrochloride seem to indicate that the administration of this drug in the amounts used and over the periods tested had no appreciable effect on *Opisthorchis* infection. The effect of chloroquine phosphate administered by the same route has been studied in 57 patients. Viewing the several series as a whole (Figure 1), the following facts deserve careful consideration: in the first place, it seems that shortly after administration, the drug stimulated the flukes to egg laying in excess of normal. Whereas before treatment only one-third of the patients had egg counts higher than 5,000 eggs/gm., three to four days after treatment, over one-half of them passed eggs in excess of that amount. That this phenomenon was not due to the fact that a new batch of flukes had reached full egg laying capacity after initiation of treatment is demonstrated not only by the sudden simultaneous increase in egg counts in most patients but also by the fact that in many patients this rise was followed a few days later by a sharp decline in eggs/gm. A similar phenomenon was observed in cats and dogs by Faust and Khaw following treatment of *C. sinensis* with mercurochrome and gentian violet. However, in the present studies, the majority of eggs did not appear to be immature and imperfectly formed as reported by the above authors. Five to seven days after beginning of treatment,

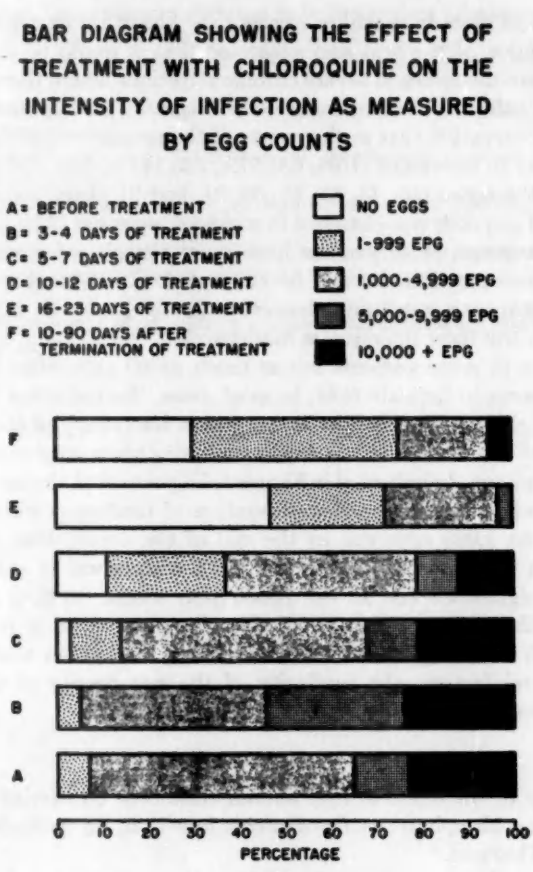
BAR DIAGRAM SHOWING THE EFFECT OF TREATMENT WITH CHLOROQUINE ON THE INTENSITY OF INFECTION AS MEASURED BY EGG COUNTS
A = BEFORE TREATMENT
B = 3-4 DAYS OF TREATMENT
C = 5-7 DAYS OF TREATMENT
D = 10-12 DAYS OF TREATMENT
E = 16-23 DAYS OF TREATMENT
F = 10-90 DAYS AFTER TERMINATION OF TREATMENT
NO EGGS
1-999 EPG
1,000-4,999 EPG
5,000-9,999 EPG
10,000 + EPG
F
E
D
C
B
A
0 10 20 30 40 50 60 70 80 90 100
PERCENTAGE

FIG. 1

an average reduction of eggs in the feces of most patients was already noticeable. At this time, 14 per cent of the patients were either negative or had less than 1,000 eggs/gm. (Figure 1). This reduction of eggs in the feces was progressively more evident as the drug was administered until at the end of treatment (16 to 23 days after the first administration of chloroquine) 47 per cent of the patients were negative. In light infections, marked reductions in the number of eggs were effected more readily than in heavy infections.

However, the adverse effect on the egg production of the worms was not always a sequel to the administration of chloroquine. In several heavy infections and a few light ones, a certain percentage of worms appeared to resist the effect of chloroquine. This phenomenon has been observed by several authors in the course of their attempts to treat *C. sinensis* with various therapeutic agents (Phan Huy Quat, 1936; Faust *et al.*, 1927). These authors pointed out the diffi-

culty that drugs have in reaching worms that "have become pocketed off" by the tissue reaction of the host and suggested that it would be much more difficult to eliminate the flukes in severe chronic infections where there was extensive liver pathology than in recently incurred infections. This hypothesis is strengthened by the observation that in the course of the present study the patients who did not respond to treatment (10H, 9A, 37A, 3A, 141N, 38S, 1388S) were among the oldest of the series (56, 43, 35, 40, 50, 21, and 21 years, respectively) while the most rapid response was obtained in a child 6 years old (22H). It is presumed that, on the average, older persons have more chronic infections with greater amount of tissue reaction. It may be concluded with Churchman (1925) that "no chemotherapeutic substance, however effective, may be expected to save cases in which the focal infection is inaccessible".

Observations of some patients for as much as 90 days after treatment was discontinued seem to indicate that, in most cases, the reduction in egg production following chloroquine therapy is not just a temporary phenomenon due to the stunning of the flukes or to a sterilization taking place only as long as the drug is present in the liver. As indicated in Figure 1, 75 per cent of the patients observed between 10 and 90 days after discontinuation of treatment were still negative or had less than 1,000 eggs/gm. at the end of the observation period. On the other hand, in two patients (272S and 346S), withdrawal of chloroquine coincided with a significant rise in egg production similar to that present before treatment. Although, on the basis of the present studies, it is impossible to determine with certainty whether the rise in egg production was due to a true relapse or to reinfection, the similarity of the egg counts to those observed before treatment seems to favor the former possibility.

SUMMARY

In the study of the effect of oral administration of quinacrine hydrochloride and chloroquine phosphate on *Opisthorchis* infection, 65 patients were treated in Northeast Thailand.

Quinacrine in the amounts used and over the periods tested failed to have any appreciable effect on the infection. On the other hand, among the patients treated with chloroquine, a marked reduction in the egg laying capacity of the worms was frequently observed as a result of treatment. In 30 out of 32 patients treated with chloroquine for 16 consecutive days or longer there was a decrease or a disappearance of *Opisthorchis* eggs in the stools. Observations of 23 patients up to 90 days after treatment was discontinued seem to indicate that, in most cases, the reduction in egg production is not just a temporary phenomenon due to stunning or to transient sterilization of the adult flukes. Significant toxicity was not observed during this study.

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TREATMENT OF MULTIPLE INTESTINAL WORM INFECTIONS WITH PIPERAZINE CITRATE

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Many persons have now confirmed the ascaricidal property of piperazine hexahydrate, first reported by Fayard in 1949. Still to be determined, however, are: (1) the shortest course or smallest effective amount in ascariasis; and (2) the cure rate in treatment of other intestinal helminth infections. In order further to elucidate the status of this drug in these regards, it was employed by us in 95 adults suffering from multiple intestinal worm infections in Honduras.

MATERIAL AND METHODS

Our studies were conducted in the United Fruit Company (Tela Railroad Company) hospital at La Lima, near the north coast of Honduras, and in two adjacent banana labor camps. The 95 men treated were all laborers on an hourly wage scale who could not afford to take any time off for treatment. Drug administration had to be geared to this fact and therefore took place in the early morning before work and/or in the afternoon after work. Eighty-five of the men had infections with *Ascaris lumbricoides*, 74 with *Necator americanus* and 48 with *Trichuris trichiuris*.

Two dosage forms of piperazine citrate were used, a syrup (either Parazine® or Antepar®) and soft gelatin capsules (Parazine®). One teaspoonful (5 ml.) of either brand of the syrup is equal to one capsule, both containing the equivalent of 500 mg. of piperazine hexahydrate. The specific dosage schedules, number of infections treated on each schedule and the length of treatment in days are noted in Tables 1 and 2. No fasting or purging were employed, either before or after treatment, on any of the schedules.

In all instances, post-treatment fecal examinations were made 10 days after completion of treatment and again two to four weeks after treatment. Egg counts were not done. The sole criterion for cure was the complete absence of eggs from direct smear and from concentrated (zinc sulfate flotation) specimens on both of the post-treatment studies.

RESULTS

The results of treatment are presented in Tables 1 and 2. The ascaricidal effect of piperazine is outstanding, whether in syrup or capsule form, whether given twice daily or in a single daily dose, even when administered for as short a period as 3 days. On all schedules, the over-all cure rates for ascaris are better than 90 per cent. In most instances, patients began to pass the worms on the day after treatment started, the majority of these worms being still alive.

In contrast to the result in ascariasis, the effect of piperazine against hookworm

TABLE 1

Treatment of multiple intestinal worm infections with syrup of piperazine citrate

Dosage Schedule	Days of Treatment	Ascaris		Hookworm		Trichuris	
		Number of infections	Per cent cured	Number of infections	Per cent cured	Number of infections	Per cent cured
10 ml. piperazine citrate (Antepar®) twice daily, at 6:00 a.m. and 3:00 p.m.....	6	16	93	7	0.0	3	33.3
20 ml piperazine citrate (Parazine®) in single daily dose at 3:00 p.m.....	6–10 (Average 8.9)	18	100	15	0.0	12	25.0
Total and averages	—	34	97	22	0.0	15	26.4

TABLE 2

Treatment of multiple intestinal worm infections with capsules of piperazine citrate

Dosage	Days of Treatment	Ascaris		Hookworm		Trichuris	
		Number of infections	Per cent cured	Number of infections	Per cent cured	Number of infections	Per cent cured
4 capsules (2.0 gram) of piperazine citrate (Parazine®, S. J. Tutag & Co.), given in single daily dose	3	16	93.7	15	6.6	8	0.0
	5	18	88.8	16	6.3	10	20.0
	6	17	94.1	21	4.7	15	26.6
Totals and averages		51	92.1	52	5.7	33	15.1

is negligible. Against *Trichuris*, piperazine is not of value when given for only 3 days, but is moderately so (25 per cent cures) when continued for 6 to 10 days.

Toxicity, although looked for, was not observed in any of the 95 adults treated.

COMMENT

Our recent publication on ascaris treatment studies in northwestern Honduras (Hoekenga, 1954) emphasized the lack of a satisfactory ascaricidal agent in this area. Of the various single-dose treatments, hexylresorcinol had given the best results with only 42 per cent cures. Of the several multiple dose schedules, diethylcarbamazine (Hetrazan®) in the amount of 12 mg./pound/day for 4 days was the most promising, with 80 per cent cures. The present study has not changed the fact that there is no good agent for a single-dose treatment. However, we believe that piperazine has now become the preferred multiple-dose anthelmintic since it gives a better than 90 per cent cure rate when administered in a single daily dose for only 3 days. In addition to its efficacy and its low cost, piperazine is highly palatable and, as noted, required neither fasting nor purging for its effective use. These are valuable properties, since they allow immediate

treatment even in debilitated patients with serious illnesses as well as in infants, and since treatment is possible without any interference in daily activities. It is convenient, too, that the liquid syrup and the capsules of piperazine are equally effective in ascariasis.

It is unfortunate that piperazine is not of significant value against hookworm and trichuris infections, because in many areas of the world two or more nematode species commonly occur in the same human host. The ideal vermifuge will be effective against all species. But, on the other hand, it is generally agreed that when the presence of hookworm eggs in the feces is not accompanied by other evidence of hookworm disease, treatment with an anthelmintic is frequently unimportant because the worms will be spontaneously excreted in time. It is also generally agreed that treatment of *Trichuris* infections is unimportant except when the infection is very heavy. An "all-purpose" vermifuge would be desirable, therefore, but frequently not truly necessary even in multiple infections.

The performance of egg-counts or the employment of new stain-preservative techniques such as MIF might have yielded more positive stools after treatment. However, we feel that we are probably getting equal results by insisting on two post-treatment stools rather than one, and by carefully examining these both by direct smear and zinc sulfate flotation.

SUMMARY

Ninety-five Honduran laborers with multiple intestinal worm infections were treated with piperazine citrate liquid or capsules. Eighty (94 per cent) of 85 men with *Ascaris lumbricoides* infections were cured, many of them on only 2.0 grams in a single daily dose for 3 days. The drug seemed to have little effect against *Trichuris trichiuris* in the dosages employed, and no effect against *Necator americanus*. No toxicity was observed.

ACKNOWLEDGEMENTS

Gratitude is expressed to S. J. Tutag and Co. (Detroit, Michigan) for supplying syrup and capsules of Parazine® and to Burroughs Wellcome and Co. (Tuckahoe, New York) for supplying syrup of Antepar®.

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WATER MANAGEMENT PLANNING FOR MALARIA PREVENTION IN THE DAMODAR VALLEY, INDIA

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While serving as a field consultant on malaria control with the Southeast Asia Regional Office, World Health Organization, in 1949, the writer reviewed multi-purpose water impoundment projects and irrigation systems over the greater part of India, including those of the Damodar Valley Corporation (hereafter referred to as D. V. C.). On a second visit in a similar capacity during 1953 with what is now the Foreign Operations Administration, U. S. Department of State, 2 months were devoted to a resident study in the D. V. C. area. His services on both engagements were performed under the auspices of the Malaria Institute of India and the Central Board of Irrigation and Power. This article is based on his findings and observations while on the foregoing missions.

GENERAL DESCRIPTION

The D. V. C. is known as "India's Tennessee Valley Authority". It is a governmental corporation created in 1948 by agreement between the National Government of India and the states of West Bengal and Bihar, in which the development is located. Unlike the Tennessee Valley Authority, the D. V. C. is mainly supported by State, rather than National, appropriations.

The D. V. C. program is multi-purpose. Its greatest single purpose is flood control, but hydroelectric production, irrigation, and navigation are all important and large-scale. Malaria control has been cited as the fourth major objective in order of priority. Secondary interests include village resettlement, land reclamation, soil conservation, domestic and industrial water supply, introduction of improved agricultural techniques and the promotion of new industries in the interest of general economic betterment and to provide an outlet for electric energy produced by D. V. C.

The D. V. C. development area extends from 10 to 260 miles northwest of Calcutta along the banks of the Damodar River and its tributaries (Figure 1). The lower part of the area, in which the irrigation system is to be located, is flat to slightly modulating alluvial plain, while the upper part containing the impoundments is rolling plateau with red lateritic soils formed by the uplifted peneplain. The population of the D. V. C. area proper is estimated at 7 million, of whom about 3 million live in the lower valley which contains the proposed irrigation system. The remaining 4 million live in the middle and upper valleys, principally in the vicinity of coal fields in the industrialized middle valley. There is a large additional tributary population, including that of metropolitan Calcutta, India's largest city.

The scale of the D. V. C. program is intermediate among the projects com-

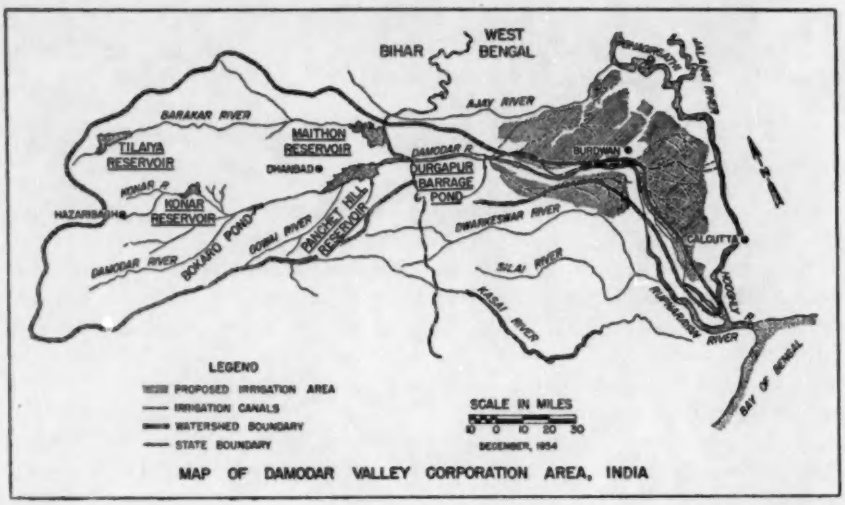

FIG. 1

prising India's current vast water resources program, but it is the most comprehensive of all in scope. The D. V. C. water resources development program is to be completed in two stages. The first stage comprises a million acres of irrigation, four multi-purpose reservoirs, two barrage ponds, a major thermal electric plant, a power transmission network, and access highways to the six impoundments. The main irrigation trunk also will provide facilities for barge navigation and will benefit the Calcutta municipal water supply. The second stage will comprise supplemental reservoirs for additional multi-purpose benefits. The estimated cost of the first stage is about $92 million, with completion by 1957. The estimated cost of the second stage is about $73 million, with indefinite starting and completion dates.

Malaria control policy. The past incidence of malaria in the greater part of the D. V. C. area has been high and still was in the year preceding the writer's last visit. While it has been postulated that some anti-malaria benefit may result from irrigation development in part of the area, it is evident that the overall plan of hydrologic development does not provide an adequate answer to the pre-existing malaria problem. For this reason, area-wide DDT residual house spraying for malaria control is indicated, and plans for extensive operations were being laid in 1953. In view of this the question might well be asked, why carry out malaria-preventive methods in the D. V. C. program of hydrologic development, especially in view of India's limited economic resources?

The basic answer to this question is that the D. V. C. water development system has been designed for a minimum life of a century and there is no present certainty that the use of inexpensive residual insecticides will permanently solve India's malaria problem. On the other hand, large capital and operating outlays to sanitize D. V. C. property holdings to an ultimate degree would seem

FIG. 2. The Damodar River in the dry season—West Bengal

to be unwise in the general setting. Hence, the policy was recommended of carrying out measures which were relatively inexpensive and enduring or which could be economically justified for other reasons.

The malaria transmission season. The climate of the Damodar Valley is tropical monsoon and the year may be divided into three seasons: 1) the cool season—November through February; 2) the hot season—March through May, and 3) the rainy season—June through October.

The main malaria transmission season is considered to be July–November for the upper valley and July–December for the lower valley. Both periods reflect the immediate effects and the early after-effects of the southwest monsoon period (June–October). The following mean precipitation figures of the weather station at Chas Road demonstrate the marked seasonal distribution of precipitation in the area:

	Precipitation in Inches
November–February	1.57
March–May	4.55
June–October (S. W. monsoon)	43.54
Annual total	49.66

The March–May period is drier than precipitation figures indicate because of the high evaporation rate. Mean daily temperatures vary from 88° F. in May (with maxima of 115°–120° F. in May and early June in Bihar), to 67° F. in January. Relative humidities which are very low during the hot dry season climb during the early monsoon period to a peak in August, the wettest month, when the average daily figure is 89%. Thus, the monsoon rains not only provide aquatic habitats but favor increased longevity in the adult mosquito by displacing extreme heat and dryness with a warm, humid climate.

Aside from the July–November and July–December periods, malaria transmission may not occur at all or may occur at lowered levels during most of the remaining months of the year, depending on locality and climate. There is wide variation in seasonal climate from year to year. Malaria transmission tends to occur throughout the year in the low alluvial plains near the coast, and to be more sharply confined in duration at the headwater Konar and Tilaiya Reservoirs, where the elevations above sea level are 1454 and 1220 feet. For various reasons, there is no consensus of opinion among malariologists on most particulars pertaining to off-season malaria transmission in the area.

The vector biological setting. A striking feature of India is the richness and diversity of the anopheline fauna. Although India is only about 40 per cent as large as the continental United States, 46 species and subspecies of *Anopheles* have been identified, of which at least 6 are recognized as major vectors of malaria and many more as lesser or probable vectors. The local importance of these vectors is determined by their density, spatial and seasonal distribution and by the complicating factor of biotypes. Two or more principal vectors may co-exist in a single local control area. There are also ecological transition zones in which one principal vector gradually gives way to another, as determined by physiographic change.

The D. V. C. area is such an area, with *Anopheles culicifacies* recognized as the major vector in the upper part of the territory, and *Anopheles philippinensis* in the lower part, with the middle part constituting a zone of contest between the two species. Among the secondary or suspected vectors in the area are *Anopheles pallidus*, *Anopheles ramsayi*, *Anopheles annularis* and *Anopheles stephensi* var. *mysorensis*.

The status of *Anopheles fluviatilis*, a primary vector in some other parts of India, is not clearly defined in the upper part of the D. V. C. area. It is considered by some that it may be the primary vector in the low level malaria transmission season and a secondary vector toward the end of the high level season. Seasonal prevalence factors prevent it from being assigned a role of greater importance. It is not present in the middle and lower zones.

The presence of a transition zone where *A. culicifacies* and *A. philippinensis* overlap between the reservoir area and the irrigation area and extending into the near margins of both, introduced important complications in the planning of antimalaria preventive measures. The introduction of new aquatic habitats and the alteration of local climates by water resources development might well extend the spatial and/or seasonal distribution of any or all vectors. Preventive

measures against the principal indigenous vector could set the stage for the introduction and high prevalence of the other nearby major vector. Because these issues could not be fully settled on the basis of available information, carefully selected ecological field studies assumed an important position in the proposed plan of malaria prevention.

THE FOUR RESERVOIRS AND TWO BARRAGE PONDS

The four main reservoirs and two barrage ponds in the first phase of D. V. C. development will form an integrated water-use and water-management network. In combination with the thermal electric plant, they also will form an integrated power grid (Figure 1).

Beginning with the upstream impoundment in each case, Tilaiya and Maithon Reservoirs are located on the Barakar River, a main fork of the Damodar River. Konar Reservoir is on the Konar River, a small tributary which discharges into the Damodar River above Bokaro Pond and Panchet Hill Reservoir. The confluence of the Barakar and Damodar Rivers is upstream from Durgapur Pond, where the headgates of the irrigation system are to be located. Thus, from a water management standpoint, Tilaiya and Maithon Reservoirs constitute one integrated series, while Konar, Bokaro and Panchet Hill Reservoirs form another integrated series, and the two separate sub-systems are in series-parallel with respect to Durgapur Pond in that one chain can be used either to augment or to substitute for the flow of the other chain into Durgapur Pond.

Prior to the writer's return visit in 1953, individual hydrographs, water management schedules and water balance accounts had been prepared by the engineering staff and consultants of D. V. C. for each reservoir and for the system. These were prepared for 17 individual water years and for the highest observed historic flood flow and were based on detailed flow records for the water years 1933–34 to 1949–50 plus long-term flood records. All of the many factors in the multi-purpose program had been taken into account, other than malaria control. These included flood control, irrigation, navigation, hydroelectric, industrial water supply, desiltation and stream flushing requirements.

The principal vectors taken into consideration in the reservoir-pond malaria control plan were *A. culicifacies* and *A. philippinensis*. *A. fluviatilis* was present in some of the reservoir areas, but it was considered that impoundment would reduce its prevalence within the submergence areas. *A. culicifacies* is a ubiquitous breeder in sunlit situations with a preference for shallow collections of water, often small in extent and temporary in character. It somewhat resembles *Anopheles albimanus* in aquatic habitat. *A. philippinensis* on the other hand is found in permanent collections of water with aquatic vegetation and closely resembles *Anopheles quadrimaculatus* in aquatic habitat. Most Indian plains rivers are dry for several months each year but become flood torrents during the southwest monsoon months. This, in combination with the flood control and irrigation purposes of the reservoirs, makes for long sustained drawdown and sharp refill characteristics The net effect of these changing water levels is to sharply minimize the malariogenic importance of the main body of the reservoir and to

elevate the importance of residual pools located in the zone of drawdown and refill, where production of *A. culicifacies* is most apt to occur. All of the reservoir sites are in well-established *culicifacies* territory.

The two minor barrage ponds, on the other hand, tend to be constant-level pool impoundments. Durgapur Pond, about 3,500 acres in size, is to be located in flat topography at a location well within the home range of *A. philippinensis*. It serves as a diversion impoundment for the irrigation and navigation canals, with consequent constant-level requirements. Extensive shallow areas favor the establishment of a permanent aquatic flora. Bokaro Pond was impounded in 1952 and its 251 acres of water surface include shallow submerged paddy fields from which *A. culicifacies* larvae have been collected. *A. philippinensis* adults also have been found in the immediate area. Its purpose is to supply 400 second-feet of cooling water to the 200,000 kw. Bokaro thermal electric plant.

As previously stated, the main malaria transmission season during normal years in the general area of the reservoirs is July through November, with a rapid fall-off in November. The related vector production season is June through October. Due to the effects of heavy monsoon rains, the four reservoirs should not present a significant problem during the early monsoon period due to the rapid rise of water level which soon submerges most of the marginal pools as well as inhibiting production in the main reservoir pools. Heavy discharges through the reservoirs in conformance with the priority objective of flood control and for desiltation, can be readily managed to provide sharp surges during this period in the two barrage ponds. After the reservoirs have been allowed to rise to their full live storage levels during the latter stages of the monsoon, they tend temporarily to become constant-level pools. It is not expected that this condition will give rise to significant production, due to the combined effects of flood surge flows, favorable terrain slopes, turbidity, clearing, waves and rain, the habits of *A. culicifacies* and especially, the submergence of residual pools. However, with declining rainfall and flows in the late monsoon period, the two barrage ponds are expected to present a substantial threat.

Based on conclusions reached from this information, the following recommendations were made.

Clearing. Clearing of all trees and brush within and immediately beyond full pool levels was recommended as standard procedure on all impoundments. It was considered that this step was fully justified on the grounds of other reservoir uses and because of the low cost due to the very light nature of nearly all of the clearing work. Certain very steep slopes with shrub growth were excepted from clearing requirements.

Residual pools. Natural depressions in the drawdown-refill zones were relatively few, but the importance of draining these, as well as of all construction borrowpits, was emphasized. The volume of work involved was limited. A very extensive residual pool problem was presented by the large numbers of small bunded paddy fields within the drawdown zone of all of the reservoirs. As a result of the heavy precipitation, rice is cultivated in these fields by inundation during the southwest monsoon period. The fields, located on gentle to steeply

FIG. 3. Hillside paddy fields—Bihar State

sloping ground, and averaging about one-tenth acre in size, are bordered by earth dams about a foot in height which retain the surface runoff for wet rice culture. The longevity of these bunds or bench terraces, after intermittent submergence within the reservoirs, could not be reliably predicted. If preserved, they could trap retreating reservoir waters at various times throughout the year depending on the water-year hydrographs. In abnormal years they could also retain rainfall for appreciable periods of time in the spring and summer before submergence by rising reservoir waters. For these reasons it was recommended that breaks be made in all bunds as a part of reservoir site preparation.

This recommendation resulted in a substantial conflict of opinion with D. V. C. land resettlement and agricultural personnel, who had planned to grow crops on the emerging terraces during the "Rabi" or dry season, as contrasted from the "Kharif" or wet season. The Rabi planting period is November to December 15, and the growing season lasts through March. During this period, crops with very low water duties may be grown provided they are planted in the dry sandy beds of rivers where ground water is near the surface or where limited quantities of irrigation water can be supplied. The principal types of Rabi crops are oil seeds and pulse (certain types of legumes). It was planned to grow these crops on the lower emerging terraces and to water them with water stored in the higher emerging terraces. Commitments had been made with villagers to lease the lands to them for this purpose and it was considered the plan would recoup a substantial part of the agricultural production lost by the permanent submergence of Rabi and Kharif crop lands at lower levels.

The issue was resolved in part by recommending the pre-impoundment breaking of only those bunds which would still be submerged in the median year, one week before the latest planting date (December 15), as determined from the reservoir hydrographs. The remaining fields were to be held under observation

after impoundment to determine their anopheline production significance, with corrective measures to be taken if indicated.

Periodic fluctuation. In contrast to the four main reservoirs, substantial vector production was considered probable in the main pools of the two constant-level barrage ponds. Use requirements and dam design prohibited incorporating water level recession in the water management plans of these two shallow ponds. A plan of weekly water level fluctuation was ultimately developed which conformed to other water use requirements.

The plan for Durgapur Pond was particularly complicated, since it required integrating the water management plans of 5 upstream hydroelectric and cooling water impoundments, plus downstream irrigation, navigation and municipal water supply requirements, with the management of periodic fluctuation in this impoundment. In spite of these complications, it was found possible to incorporate a 24-hour drawdown of 1.25 to 2.75 ft. in 24 hours even under capacity head conditions in the outlet canals without wastage of usable water, and to achieve an equal rise in level spread over the other 6 days of the week. Adjustment of inflow was made possible largely by the high proportion of electric energy scheduled for potential industrial use, with a consequent prolonged off-peak period during each week-end, and by the flexibility which the 200,000 kw. thermal electric plant provided in the power grid.

At Bokaro Pond, due to the very limited live storage in upstream Konar Reservoir, development of a feasible drawdown-refill cycle was made possible by recirculation of the 400 second-feet of cooling water required by the thermal plant during the 6-day refill period and by discharging it on the weekly drawdown day.

Permanent work. The only permanent work recommended in the total plan which was directly chargeable to malaria control account was the placement of 78,000 cubic yards of shallow earth fill for shoreline improvements at Bokaro Pond. This step was recommended because of the malariogenic importance of the areas involved and the permanency and importance of the adjacent multi-million dollar Bokaro Camp and thermal plant.

THE IRRIGATION SYSTEM

Irrigation system plans. The entire irrigation system is to be located in West Bengal and will be served by two main canals located on opposite sides of and roughly paralleling the Damodar River. The canal headwaters are at Durgapur Pond. The larger and longer of the two main canals will have a total length of 83 miles and will serve as a navigation canal for barges and to supply water at the point of intake of the Calcutta water system, as well as for irrigation. It will take in a smaller existing irrigation system known as the Damodar-Eden Canal system, which presently supplies water to a more limited area in the upper part of the proposed system.

Water is to be supplied for two crop seasons—the Kharif or monsoon season (June–October), and the Rabi or dry season (November–March). The purpose of the Kharif season irrigation is to supply only supplemental water for rice, in

FIG. 4. Damodar-Eden main irrigation canal—West Bengal

view of the heavy rainfall in that season. The principal need for this water will be in the declining phase of the monsoon. The purpose of the Rabi season irrigation is to supply a primary water supply to dry crops, in view of the virtual absence of precipitation in the Rabi season. The maximum irrigation water demand is during the Kharif season in October and is expected to amount to 8,206 second-feet under full development. The crop areas involved in round figures are 1 million acres in the Kharif season and 300 thousand acres in the Rabi season. The maximum supplemental water duty in the Kharif season is 24 acre-inches, spread over 4½ months, added to an average rainfall for the period of 46 inches. The primary water duty in the Rabi season is 18 acre-inches, spread over 5 months.

Malaria survey results. The potential man-made malaria hazard presented by the six impoundments is considered rather inconsequential compared with that of the proposed million acre irrigation system. The population of the general area to be irrigated is about 3 million. The number of people who actually will be exposed to malaria risk has not been estimated, but it was stated that over 16 hundred villages and towns would be directly affected. The population per community varied from 30 to 3,905 people in 140 of these communities which were surveyed for malaria.

Recent reliable malariometric data for on-season rates are lacking for the area, but an off-season survey of 140 villages totaling some 52 thousand people was made in January and February 1952. The area involved was in the upper part of the irrigated area and the age range of the group examined was 1 month to 10 years. *Vivax* and *falciparum* malaria were both present. The parasitemia rate was 20.4%, varying from 0 to 55% per village. The splenomegaly rate was 28.5%, varying from 0 to 86% per village. There were two villages which had high splenomegaly rates but zero parasite rates, suggesting interference from

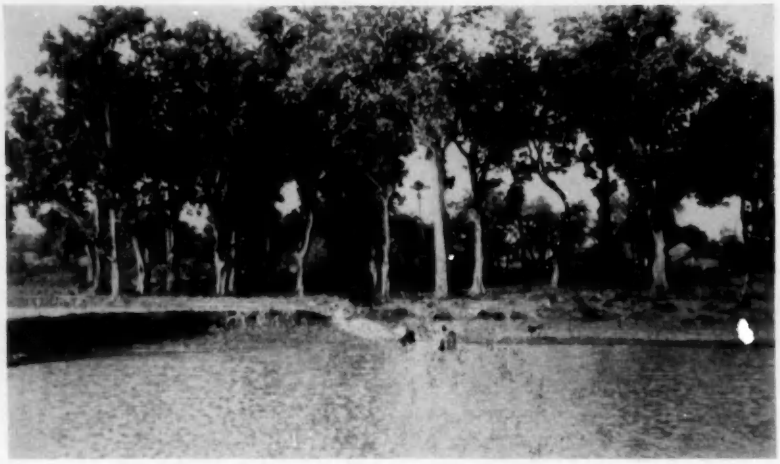

FIG. 5. A tank in West Bengal

other indigenous infections such as kala-azar, but a total of only 13 blood films were collected from these two villages.

A portion of the proposed irrigated area is notorious even in India for its high malariogenicity and has been cited on various occasions in the popular and professional literature as an example of social decay and depopulation directly attributable to malaria. On the other hand, portions of the lower part of the area have been nearly free of malaria, although located in far more poorly drained terrain.

The malaria vector. Deltaic West Bengal is known as "tank" country. Tanks are round, artifically excavated earth reservoirs which collect water by surface runoff from the immediately adjacent area during the monsoon season and which hold water continuously in normal years. Located in impervious clay subsoils, they constitute perched water tables in the upper part of the area. In this area most tanks are community affairs, with one or two being found at each village. They are vital to the life of the village, and serve as sources of domestic water supply, animal wallows and bathing and washing places.

In the lower part of the area approaching Calcutta, the tanks become more numerous and smaller, and serve individual houses or a few houses, rather than an entire village. Due to the higher water table in the lower area, they merge into the ground water table and appear to be largely supplied with water from this source. Often centuries old, they frequently are rich in aquatic flora and possess the general appearance of small lime sinks. The confirmed, principal vector in the general area is *A. philippinensis*, and it is almost exclusively a tank breeder in deltaic West Bengal. The larvae are found principally in tanks containing aquatic vegetation, provided the surface is not occluded as by rank growths of water hyacinth.

Plans for irrigation development have generated two diametrically opposed malariologic concepts. It is postulated by some that *A. philippinensis* production

is inversely proportional to the height of the water table. This case is supported by evidence that in the lower part of the area, where the water table is near the ground surface, *A. philippinensis* production in tanks and malaria rates are both low. In the upper part of the area the reverse takes place. In accordance with this concept, every possible means should be taken to raise the ground water table up to the point of land overflow. Excess irrigation water should be applied to the land at every possible opportunity and drainage should be avoided.

Others postulate that *culicifacies*, which is already established in the area in small numbers, would become firmly established by such practices without any assurance that *philippinensis* would be done away with, the net result being a great increase in malaria. This is based on the high prevalence of *culicifacies* in irrigated areas in other parts of India. Minimum water application, maximum drainage and other conservation irrigation measures are consequently recommended toward the prevention of *culicifacies* production.

The aquatic biologist will readily appreciate the many factors which may and probably do, enter into the tank breeding picture, of which ground water table elevation per se is one. Engineers equally will appreciate the uncertain effects on the ground water table of applying the limited quantities of irrigation water involved under varying conditions of soils, terrain, ground water level and precipitation.

Five intensive field studies were recommended toward the solution of this dilemma and related malariologic problem. Protocols for these were prepared. The least important study consisted of additional mosquito dissections toward identifying and evaluating secondary vectors. A second study was to evaluate the postulated increase in prevalence of *A. culicifacies* in the proposed irrigated area which will take place following the development of irrigation under normal practices. Opportunity for carrying out this study was provided by the existing Damodar-Eden irrigation system and by the selection of an appropriately paired unirrigated area. A third comparison study in the same paired areas was intended to measure the effects of irrigation on the ground water table in the terrain phases involved. The purpose of the fourth study was to obtain base line information on ground water tables and perched water tables throughout the entire area proposed for irrigation, especially during the monsoon season.

The fifth study was a multi-professional undertaking involving hydrologic-limnologic-entomologic observations and was for the purpose of obtaining basic ecological information on *A. philippinensis* production in tanks located in non-malarious areas versus those in malarious areas, as established by malariometric surveys. This study, in combination with the hydrologic studies, represented an effort to evaluate the postulated direct cause and effect relationship between the water table and malaria and the effect of various possible irrigation plans and practices on this relationship.

SUMMARY

The current and proposed plan of development of the Damodar Valley Corporation, often referred to as India's T. V. A., is outlined. Malaria survey results are reported for the pre-development stage. Recommendations which were made

toward malaria prevention in the vicinities of six artificial impoundments and in a million acre area to be irrigated are described. The malariogenic importance of the irrigation development is considered to be far greater than that of the reservoirs. Two conflicting opinions as to the measures which should be undertaken to curb "irrigation malaria" in this particular area, are presented, and a proposal for field research in anopheline ecology is described. The objectives of this research are to evaluate the potential problem and to provide a basis for developing appropriate counter-measures.

THE DENSITY OF ADULT ANOPHELES IN THE NEIGHBOURHOOD OF AN EAST AFRICAN VILLAGE[1]

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During the course of the years 1951–1953, observations were carried out in some detail on certain aspects of the adult behavior of these two *Anopheles*. These studies were mainly concerned with the distribution of the daytime resting population in houses and outdoor shelters, with the duration of the gonotrophic cycle and with the relation of blood meals to ovarian development. The results of this work have been published elsewhere, but certain data and conclusions presented in them need to be considered here, as a preface to the present account of an investigation into the density of these species in the East African locality concerned. It should be noted that the work reported here was carried out as an integral part of the other studies referred to.

The following conclusions should be recorded:

(1) Both *A. gambiae* and *A. funestus* are almost wholly anthropophilic in this district. Cattle are absent, owing to the infiltration into the area of *Glossina*, and wild mammalian hosts are evidently of negligible importance. The house resting population as studied by Davidson and Draper (1953) in another village in the same district, was shown to have an anthropophilic index of 100 per cent. The outside resting population has been found to consist mainly of unfed and gravid females (Gillies, 1954*a*). Of the few fed females caught outside, practically all were found by precipitin tests to have fed on man.

(2) About 5 per cent of both species rest outside after feeding (Gillies, 1954*b*).

(3) The gonotrophic cycle in both species at mean temperatures above 78°F. lasts 48 hours (Gillies, 1953). Females collected in the daytime can be classified, therefore, as freshly fed, gravid or unfed.

(4) In the resting and digesting phase of each cycle, as opposed to the host seeking and oviposition phases, the mortality is likely to be very low or negligible. There is some evidence (Gillies, 1954*b*) to support this supposition, and the work of Davidson and Draper (*loc. cit.*) suggests that even in the course of one complete gonotrophic cycle the mortality may only be of the order of 14–16 per cent. The number of gravid females in the whole population may be expected, therefore, to fall not far short of the total of fed females.

(5) Data on the distribution of unfed and gravid females in houses and outside shelters has also been accumulated (Gillies, 1954*a*,*b*).

From these observations, it is clear that the total numbers of freshly fed females resting in all the houses in the area in the daytime comprise the over-

[1] This work was carried out under the administration of the East Africa High Commission. The funds were provided by the East African Governments and by the Colonial Office from the research allocation of the Colonial Development and Welfare Acts, acting on the recommendation of the Colonial Medical Research Committee.

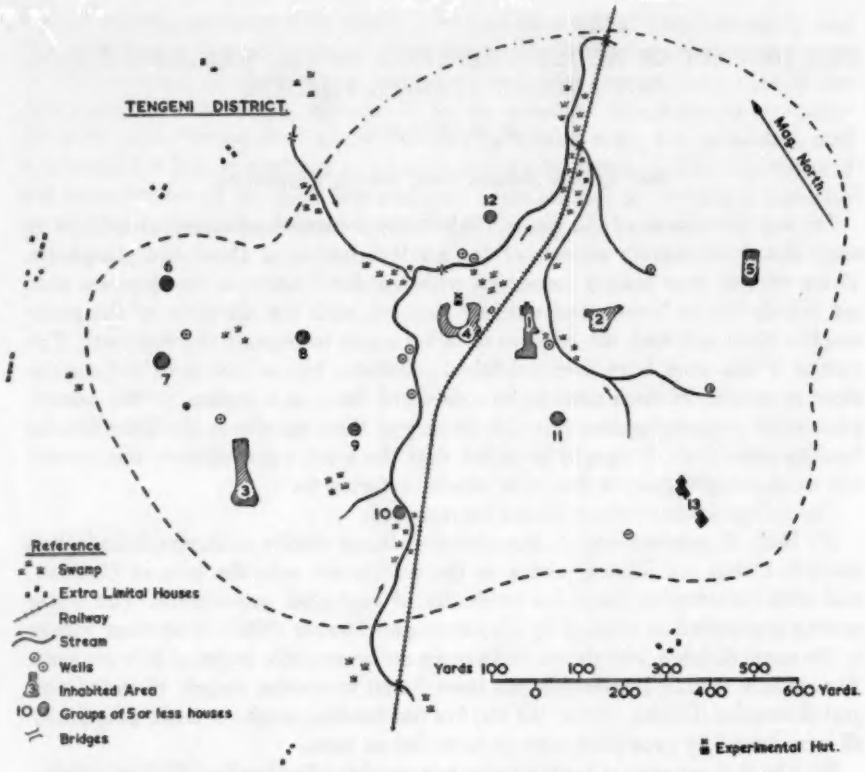

FIG. 1. Map of Tengeni showing the distribution of houses. The interrupted line illustrates the limits of the section within which spray catching was carried out.

whelming majority of the biting population of the previous night. The fed section of the mosquito population could be collected by spray-catching in the houses and counted, and from this figure the total numbers present in the area, at all stages of ovarian development and activity, could be estimated with reasonable confidence.

This paper records the results of such an investigation in and around the village of Tengeni, 26 miles inland from the East African coast at Tanga (lat. 5° S.), some account of which has already been given (Gillies, 1954*a*). To this one should add that Tengeni forms part of a continuous belt of little clusters of houses set on the high ground and of wells and seasonal swamps in the valley bottoms, so that it is often impossible to define where one village stops or the next begins. Thus it was decided to delimit one section of this inhabited belt and to collect all the mosquitoes that were feeding, on one night, on the human inhabitants of the section. The limits of the section were taken as extending, at any point on the perimeter, half way to the next group of houses, on the assumption that hungry mosquitoes would in general fly to the nearest source of

blood. In Figure 1, the sprayed section round Tengeni is shown together with the nearest groups of houses outside the section and with the perimeter of the area served, as far as the mosquitoes were concerned, by the sprayed houses. This limit is arbitrary along the north-eastern boundary of the section, as in this direction the next group of houses was a mile and a half away; but on all other sides the groups of houses were distributed at regular intervals and the perimeter represents, as accurately as possible, the halfway line between sprayed and unsprayed houses.

Method of investigation. A circle with a radius of 700 yards round an experimental hut, more or less in the middle of the village, was chosen as the limits of the section of countryside to be investigated. Within this area there were five large and eight small but distinct groups of houses (Fig. 1), separated by distances ranging from 100 to 300 yards. In these groups there were 119 inhabited houses and one experimental hut in which two paid volunteers slept. It was obviously impossible to spray all the houses on one day, and so the catches were made on 5 days spaced out over an 11-day period. (At the end of this period, 3 houses remained outstanding and were sprayed separately on the 12th day.) An interval of either 2 or 3 days was left between the catching days, and it was hoped that this would be sufficient to allow infiltration of mosquitoes from the immediately surrounding district on a large enough scale to replace the estimated 12–15 per cent of the local population removed by each spray catching. By this means, the aggregate of the catches made on the 5 spraying days could be regarded as yielding the equivalent of all the mosquitoes resting in the houses of the village on any one day.

The area was divided up, for catching purposes, into five zones of 23–25 houses each, and a spray catching team was allotted to each zone. Five houses in each zone were sprayed on each catching day, and to randomise the catches further, the houses were numbered and every fifth house was selected for spraying on the same day. Thus not more than one house in each group of five was to be sprayed on the same occasion. In practise, owing to the occasional absence of the occupants from their houses on the day allotted for spraying, some departures from this plan were necessary. Each team consisted of two sprayers under the charge of a European Field Officer or an African Malaria Assistant. Pyrethrins in kerosene (0.1 per cent) were employed and the teams were supplied with sufficient supplies of cotton sheeting to cover all the floor space and horizontal surfaces of every room in each house. Any gross defects in the walls were also covered with sheeting.

The whole of a house was sprayed at one time, and where, as happened in a minority of cases, the floor space was too big for one team to cover, additional teams were called in to help so that in one or two instances 6 or 8 sprayers were operating simultaneously in, and around the eaves of, the same house. It was felt that, by this means, an effective kill of a high percentage of the mosquitoes resting in every house in the district was obtained; in only one house, the height and floor space of which were exceptional, was an inadequate coverage with insecticidal spray obtained.

After spraying, the mosquitoes were picked up and placed in flat cigarette

tins, which were lined with damp lint. The specimens in each tin were sorted on return to the laboratory, males and culicines counted and discarded and all anopheles females dissected and the ovarian stages and degree of blood digestion recorded. It was not possible to complete the sorting of all the material in one day, but, by lightly sprinkling the boxes with 2 per cent formaldehyde, the mosquitoes could be adequately preserved for identification and staging on the following day.

The number of persons sleeping in each hut was ascertained in a preliminary survey with the chief of the village, and was again checked by interrogation at the time each house was sprayed. No attempt was made to verify the truth of these statements by nightly inspections, but, in view of the relatively large numbers of houses involved, it seems unlikely that the occasional false statements made would materially affect the validity of the calculations on the number of mosquitoes biting per person.

The investigation was carried out from the 1st to the 12th of December, 1952. Screen temperatures over this period gave an average daily maximum of 90.4°F. and minimum of 71.3°, the mean temperature being 80.8°. No rain fell during the course of the observations, but there had been heavy falls at the end of October and in the first half of November that had given rise to the relatively large mosquito populations found.

RESULTS

These are set out in Tables 1 and 2. The following points should be noted.

(1) Out of a total of 27,719 mosquitoes caught, 11,157 were freshly fed females of *A. gambiae* and *A. funestus*. This gives a density of 35.2 fed females per head of human population over the whole period. Successive catches over the four main spraying days remained constant within reasonable limits. Owing to the enormous variation in catches from house to house, the geometric mean of the number of fed females per person for each day has been calculated and is also set out in Table 2. This treatment of the data does not materially alter the conlusions drawn.

TABLE 1

Results of spray-catches in 119 houses

Date	*A. gambiae*		*A. funestus*		Other *Anopheles*		Culicines		Total
	Males	Females	Males	Females	Males	Females	Males	Females	
December 1	1231	2869	387	1740	—	2	212	201	6642
December 3	863	2274	456	1887	—	3	133	191	5807
December 6	794	2061	596	2030	—	21	286	309	6097
December 8	642	1769	855	2055	—	—	186	206	5713
December 11	276	555	746	1425	—	1	120	113	3236
December 12	9	15	37	38	—	—	69	56	224
Total	3815	9543	3077	9175	—	27	1006	1076	27,719

TABLE 2

Numbers of recently fed Anopheles *in relation to human population in houses*

Date	No. of Houses	*A. gambiae*	*A. funestus*	Both Species	Human Population	*Anopheles* per Person		Houses with Less than 4 Sleepers
						Arith. mean	Geometric mean	
								%
December 1.....	25	1968	927	2895	80	36.2	29.8	68
December 3.....	25	1543	1042	2585	72	35.9	19.8	72
December 6.....	25	1208	1060	2268	66	34.4	22.4	68
December 8.....	25	1260	1140	2400	54	44.4	23.8	88
December 11.....	16	308	612	920	38	24	17.7	81
December 12.....	3	7	13	20	5	4	3.1	
Experimental hut av.—December 1–12...		31	38	69	2	34.5	32.7	
Total.....	120	6325	4832	11,157	317	35.2	28.9	75

(2) There was a progressive change in the proportions of the two species in each successive catch, *funestus* becoming more numerous and *gambiae* scarcer. Continuous catches from the experimental hut showed that this was part of a general trend, due to the drying up of the more temporary *gambiae* breeding sites during the period of the investigation, and the rise in productivity of the semi-permanent swamps favored by *funestus*.

(3) The only other anopheles caught were very small numbers of *A. marshalli*, probably a partly exophilic mosquito. The culicines included small numbers of *Taeniorhynchus* (*Mansonioides*) and larger numbers of *Culex fatigans* and undetermined species of *Culex*.

It is well known that hungry mosquitoes are attracted to houses in densities that are related to the numbers of human occupants. But it is also probable that the relationship is not a simple arithmetical one and that the greater the number of sleepers the fewer bites each individual receives. (A clear instance of this is to be found in the data collected by Haddow, 1942.) It is shown for the present investigation in Table 3. The difference between one person and two or three per hut is not evident when the geometric mean is calculated, probably owing to the greater variation in attractiveness of a single sleeper, but in houses with more than three occupants the number of bites per person is clearly reduced. In comparing spray catches on successive days, both factors should be taken into consideration. The results in Table 2 have accordingly been expressed in terms of the numbers of anopheles per person, and as a further check on the distribution of crowded and uncrowded houses on different days, the proportion of houses with less than four inhabitants is recorded as well. From the results expressed in this way it is seen that there was little variation over the four main spraying days. The increase in numbers of anopheles per person on 8th December may evidently have been influenced by the higher proportion of houses with small numbers of occupants sprayed on that day. The lower figures for the

TABLE 3

Numbers of fed mosquitoes in relation to numbers of inhabitants

People per House	No. of Houses	Mosquitoes per House	Mosquitoes per Person	
			Arith. mean	Geometric mean
4–7	29	129.2	26.6	14.4
2–3	60	89.7	44.8	24.6
1	30	63.4	63.4	24.6

density of anopheles on 11th and 12th December are almost certainly attributable in part to the numbers of houses difficult of access and, for various reasons, awkward to spray, that were put off until the end of the campaign.

Estimation of population density. From data on the distribution of the mosquito population and on the proportions at different stages of the gonotrophic cycle, already outlined, the following calculations on the total size of the mosquito population in the area may be made.

	A. gambiae	*A. funestus*
Spray catch, fed females	6,325	4,832
5 per cent of total, resting outside	332	254
Total fed	6,657	5,086
Total gravid	6,657	5,086
Fed and gravid	13,314	<10,172
+ 17 per cent, unfed*	2,263 + 47.5 per cent, unfed*	4,830
	15,577	15,002

Thus it is seen that there was a total of about 15,000 females of each species within the section chosen. As the area within the section amounted to some 380 acres, the spatial density of each species in and around the village was of the order of 41 females of *gambiae* and 39 females of *funestus* per acre. The expression of anopheles density in numbers per unit area is admittedly somewhat artificial when applied to the house resting population, but when the outside resting population is considered, the results are quite instructive.

Density of outside resting population. Estimation of the outside resting population may be made in the following way, the source of the data on the distribu-

* Table 1, Gillies (1954*a*) and Table 3, (1954*b*); ratio of unfed to gravid *gambiae* outside was 0.55:1 and inside 0.14:1; the gravid population was found to be equally distributed inside and outside houses, hence unfed females represented 0.34 × the total gravid population, or 17 per cent × total fed and gravid. Similarly from Table 3 (1954*a*) and Table 3 (1954*b*), ratio of unfed to gravid *funestus* in outside shelters remote from houses was 2.3:1 and inside 0.39:1; the gravid population was distributed in the proportions of 30 per cent outside and 70 per cent inside houses, hence unfed females represented 0.95 × the total gravid population, or 47.5 per cent × total fed and gravid.

tion of females at different stages of ovarian development being mentioned in parentheses.

A. gambiae

Fed.	5 per cent of total fed (1954*b*, Fig. 1) 332/380	= 0.9 females/acre
Gravid.	50 per cent of total gravid (*loc. cit.*) (6,657)/(2 × 380)	= 9 females/acre
Unfed.	0.55 × total gravid outside (1954*a*, Table 1)	= 5 females/acre
Total		14.9 females/acre

A. funestus

Fed.	5 per cent of total fed (1954*b*, Fig. 1) 254/380	= 0.7 females/acre
Gravid.	30 per cent of total gravid (*loc. cit.*) (5,086 × 0.3)/(380)	= 4 females/acre
Unfed.	2.3 × total gravid outside (1954*a*, Table 3, hot season, distant shelters)	= 9 females/acre
Total		13.7 females/acre

Seasonal fluctuations in density. In the center of the village an experimental hut had been constructed, in which two paid volunteers slept without mosquito nets and in which daily hand catches were maintained for a period of over a year. While catches from a single hut cannot be accepted as bearing a constant relation to the total population of the area, it is likely that they reflect fairly accurately the general trend of population size and can be used to indicate the limits of seasonal fluctuation of the two species. Over the 52-week period from April 1952 to April 1953 the arithmetic mean of 4-weekly catches of *gambiae* varied from 1.3–36 females biting per night, and of *funestus* from 0.5–76 females per night. As, at the time of the population study recorded here, the experimental hut catches averaged 31 *gambiae* and 38 *funestus* per night, the limits of the seasonal fluctuations in density for the two species within the section studied can be calculated as between 650 and 18,000 female *gambiae* and between 200 and 30,000 female *funestus*. In terms of spatial density the annual limits of variation are 2–48 *gambiae* per acre and 0.5–78 *funestus* per acre. These limits can only be taken as approximate, but they do, nevertheless, indicate quite usefully the range of densities that may be expected to occur.

Sources of error. If the general validity of the conclusions on the distribution of the resting population is accepted, these being based on previous and contemporaneous studies, then certain sources of error implicit in the technique employed in the present investigation need further mention.

(1) While spraying with pyrethrum is certainly the most efficient method of catching mosquitoes in houses of simple construction, particularly when carried out in the systematic manner described, it is nevertheless true that a certain fraction escape. This proportion varies, perhaps, from less than 10 per cent in favorable up to 20 per cent in unfavorable houses. The error is a real one, but it should be remembered that gorged females, which formed the basis of the calculations, are notoriously sluggish and are the least likely to escape of all mosquitoes.

(2) Another potential source of error may lie in the progressive destruction

and depletion to which the mosquito population was subjected. To minimise or eliminate this effect, the days on which spraying was carried out were spaced at intervals of either two or three days. The constancy of the average numbers biting per head of human population over the four main spraying days, shown in Table 2, indicates that re-entry into the houses from outside resting sites, and infiltration from the immediately surrounding areas, was quite adequate to replace the 20 per cent of the house resting population removed by each day's spray-catching. There may possibly be some indication of depletion in the catch made on the fifth day, but this would effect only 13 per cent of the catches.

(3) The possibility also exists that the repellent effect of pyrethrum in the houses already sprayed might cause mosquitoes to feed or rest in the as yet unsprayed houses, and thereby give artificially high levels to catches made on later days. The fact that the density of anopheles per person on the first day was the same as the average for the whole period, suggests again that this did little to influence the results.

(4) It has been shown elsewhere (Gillies, 1954*c*), that in some 20 per cent of fed *A. gambiae*, maturation of the ovaries does not follow the blood meal. Thus this proportion of fed females is not matched by gravid females, and the numbers of the two groups in the whole population may not be equal. Some of these females will appear in catches as pre-gravid females, i.e. with ovaries at Christophers' stage II and with old blood in the gut, and such females in the present work have been classified as gravid. But others may feed on two consecutive days, in which case they will be counted again in the fed population and be estimated for again amongst the gravid females. In either case the error involved will certainly be less than 20 per cent.

DISCUSSION

Conditions at Tengeni offered exceptional opportunities for carrying out a relatively accurate census of the vector populations. These opportunities derived, as has been shown, from the almost complete anthropophily of the two anopheles studied and from the tendency of all except a small percentage of females to remain indoors for at least the first half of the gonotrophic cycle. It is unlikely that the limits of error from the sources enumerated above exceeded 30 per cent. As regards the basic data on the distribution of the daytime resting population, some margin of error must obviously be allowed, and the margin may be large in the case of unfed *funestus* females. But even with this qualification, a maximum error of 50 per cent in the estimation of the total population is the most that can be admitted, and the true figure may very well lie within 25 per cent of the calculated density.

It is interesting to compare these results from East Africa with those obtained in other parts of the world with other techniques. By using marking and recapture methods in Tennessee, Eyles and Cox (1943) estimated that the spatial density of *Anopheles quadrimaculatus* lay between 8,000 and 15,000 females per acre. An area of 400 acres—roughly the same size as the area chosen for the pres-

ent observations—was calculated to harbour some 3–6 million mosquitoes, which may be compared with the 10–20,000 of each species recorded from Tengeni. Even at peak seasons the *funestus* population cannot have exceeded 50,000 females. In the savannahs of Eastern Colombia De Zulueta (1950, 1952) estimated, by direct trapping of mosquitoes resting in the grass, that there were about 0.25 female anopheles per sq. metre in the open savannah and 0.1 per sq. metre in the neighbourhood of dwellings. These figures are equivalent to densities of 1,000 and 400 females per acre respectively, and represent populations that are again higher than the maximum of 50–100 per acre at peak seasons recorded from East Africa. It should be noted however that the population of *A. darlingi* was so small that the trapping methods used by De Zulueta (1952) yielded only a single specimen.

These results emphasise again the vastly different mechanisms of malaria transmission by efficient vectors in the tropics and by less well adapted anophelines in more temperate regions. The inhabitants of Tengeni, as exemplified by the two sleepers in the experimental hut, each received not less than 220 potentially infective bites in the course of a year. Of these possible exposures to infection, 127 were due to *gambiae* and 93 to *funestus*, which was more numerous but less highly infected (Gillies, 1954*c*). This phenomenal rate of transmission, which may be encountered in many of the more humid low-lying parts of equatorial Africa, is seldom approached anywhere else in the world; yet, as has been shown, the actual density of anopheles need not be large. Under these African conditions, it is on the closeness of contact between a few hundred people and a few thousand mosquitoes throughout the year that the effectiveness of the transmission depends, and it is the operation of factors responsible for bringing the two populations together that is of primary importance. They may be listed as follows:

(1) The almost complete dependence of the mosquitoes on a single host, man. This preference is partly intrinsic and partly facultative, owing to the absence of cattle and of suitable alternative hosts. The presence of tse-tse flies has, in this instance, an interesting side effect on the transmission of another human disease.

(2) Temperature and its effect on the gonotrophic cycle. *A. gambiae* feeds every other night for all except perhaps the coolest few weeks of the year. For *A. funestus* the year is almost equally divided between a two-day and a three-day biting cycle.

(3) The longevity of the mosquitoes. Evidence is accumulating—some of it at present unpublished—to indicate that under the conditions of temperature and humidity encountered, the longevity of these two vectors, particularly of *A. gambiae*, may be exceptionally high. The work of Davidson and Draper (*loc. cit.*) suggests that the daily mortality suffered by the mosquitoes may only be of the order of 7–8 per cent, and the rather higher sporozoite rate obtained by the present writer in gland dissections made over the course of a whole year suggests that the mortality in this species may be lower still (the mean sporozoite

rate of *A. gambiae* females at all stages of ovarian development over 26 fortnightly periods was 6.9 per cent, as compared with the 4.7 per cent recorded by Davidson and Draper).

(4) As in most primitive communities, the lack of protection against mosquitoes, either by nets or substantial bed coverings or by adequate housing, was very noticeable in this East African village.

(5) Lastly, one of the factors that so often increases the intensity of transmission in Africa is the presence of two efficient vectors, whose ecological requirements may be complementary to each other, so that for only short seasons of the year are adequate populations of one or other species wanting.

When this work was first started, it was puzzling to find that, despite house resting populations of a hundred or more female anopheles at peak seasons, it was only with the greatest persistence that females were caught resting outside. It was not fully appreciated then, that those indoors in the daytime were concentrated at relatively few points, while those outside houses, however large or small a proportion of the total they represented, would always be widely dispersed and infinitely harder to find. When the relatively enormous area between the houses and the number and diversity of potential resting sites are considered, it is no longer surprising that the present author and others before him, for example Wiseman and others (1939) and Muirhead Thomson (1951), have had such little, or at the most, very meagre success in finding them. This particular point was emphasised by Barber and others (1931) in Liberia. One can now add the rather more precise information that, at Tengeni at least, there are seldom more than 30–40 females per acre resting outside, and for much of the year far fewer. Taken literally, this would mean that over 100 square yards of ground would have to be searched to find a single female anopheles. Resting sites are not, of course, distributed in this regular fashion, but it is probable that only when such sites are particularly favorable to the mosquito, as well as accessible to the searcher, that any success is achieved.

In conclusion, it can be said that malaria transmission in the East African coastal lowlands is maintained by relatively low densities of anopheles, but that the exceptionally close contact between vector and host, and the high degree of longevity enjoyed by the mosquitoes, far more than compensate for this numerical disadvantage.

SUMMARY

Previous observations in an East African village had revealed that *Anopheles gambiae* and *A. funestus* were almost completely anthropophilic, and that only 5 per cent of the biting population was known to rest outside after feeding. Spray-catching in all the houses in the village was therefore used to ascertain the total size of the biting population. From this figure the total numbers at all stages of ovarian development were estimated.

Spray catches in 119 houses yielded over 27,000 mosquitoes. The total number of *A. gambiae* and *A. funestus* was estimated at about 15,000 females of each species. As the area of the investigation totalled 380 acres, the spatial density

was calculated to be of the order of 40 females per acre of each species. The outside resting population was estimated at 14–15 females per acre of each species. The annual range of fluctuation in density was estimated from observations on the biting population in an experimental hut, in which catches were made throughout the year. The density of *A. gambiae* at different seasons was estimated to vary between 2 and 48 females per acre and of *A. funestus* between 0.5 and 78 females per acre.

These results indicate that the very high rate of malaria transmission in the area is maintained by relatively small numbers of anopheles, and that factors influencing the association of the mosquito with man, and the longevity of the mosquitoes, are of primary epidemiological importance.

ACKNOWLEDGMENTS

I am grateful to Dr. D. Bagster Wilson, Director of the East African Malaria Unit, for his helpful interest in this work. I am particularly indebted to Mr. M. C. F. Butler for surveying the area and for producing the map accompanying this paper. Mr. C. D. Ramsdale and Mr. G. T. Shute gave valuable help in carrying out the work. The help of the African staff of the Unit and of students in training is also gratefully aknowledged.

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ATTEMPT TO ERADICATE ANOPHELES IN THE MALARIA ZONE OF CHILE

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The former malaria zone in Chile was confined to the Province of Tarapacá, and included the valleys and oases situated between latitudes 18° and 21° south, comprising an area of 55,000 square kilometers (Fig. 1).

The antimalaria campaign in Chile was initiated in July, 1937, and was carried out in successive stages, beginning at the city of Arica, then continuing to the nearby valley of Azapa, and finally, in 1941, gradually extending to the remaining valleys, oases and chasms in the Province of Tarapacá, which were the only endemic malaria zones in the country. The campaign was a complete success even before the application of DDT, which served only to consolidate the gains that had been made. Not a single case of autochthonous malaria has been diagnosed in Chile since April 1945 (Noé *et al.*, 1949, 1949a; Gutiérrez *et al.*, 1950).

PROCEDURE OF THE ANTI-ANOPHELES CAMPAIGN

The only species of *Anopheles* in the former malaria zone was *Anopheles pseudopunctipennis*, Theobald, 1901. Until July, 1944, the methods employed were basically antilarval and consisted of weekly applications of petroleum and of minor sanitation such as drainage of marshy lands, filling of stagnant pools and small marshes, repair of irrigation canals, control of springs, and occasionally work along certain stretches of rivers. Irrigation canals were constructed in some areas, as in the Pica oasis and in sections of the Azapa Valley. Naturalistic measures of anopheline control were also widely applied and *Gambusia affinis*, the American top minnow, was distributed in all collections of water.

With the introduction of DDT, manufactured in our country, control procedures were modified in July, 1944, and primary importance was given to the campaign against adult mosquitoes by house spraying. Even at that time, however, Dr. Juan Noé, who was directing the campaign, suggested the possibility of eradicating the *Anopheles*, and with this in mind he employed DDT also against larvae.

In the beginning, doses of 0.1 grams of DDT per square meter of surface were applied in residual house spraying. The concentration was gradually increased as DDT became more readily available in the market, until the doses reached 2 grams per square meter. At first, each region received 4 applications a year, then 2, and finally 1, as the residual DDT concentration increased to an estimated 2 grams per square meter. Table 1 summarizes some of the overall data on DDT spraying in the Province of Tarapacá from 1945 to 1953. Gammexane has also been used in this campaign since 1949.

In 1945 and 1946, spraying with DDT at 0.5 per thousand in water emulsion

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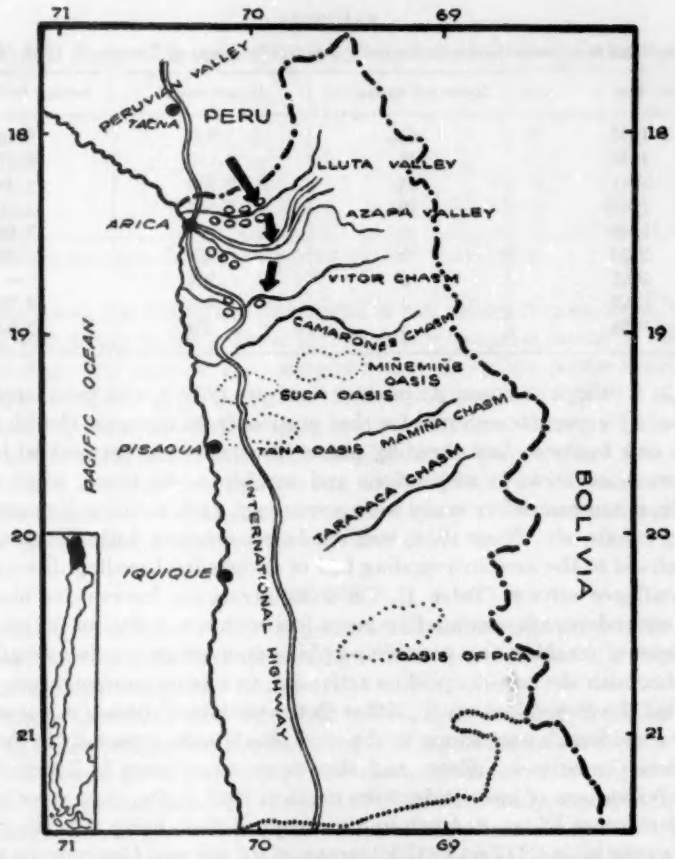

FIG. 1. Location of sectors positive for larvae of *Anopheles pseudopunctipennis* in Chile and its possible ways of spreading from north to south.

was applied on domestic animals, such as cattle, goats, horses, donkeys, and mules. These applications were made at approximate intervals of two months and they had as their purpose the destruction of the zoophilic adults of *Anopheles*, which would eventually feed on the animals. Larviciding was done with 2:1,000 and 4:1,000 DDT water emulsions.

In addition to periodic insecticide applications, an *Anopheles* surveillance service has been maintained, which makes periodic searches for larvae and pupae and tries to capture adult *Anopheles* with human or animal-baited traps in the vicinity of any larval foci discovered. The number of inspections for larvae carried out each year has been high; in 1949 there were 129,488; in 1950, 172,130; in 1951, 217,599; and in 1952, 298,713.

Antimalaria posts have gradually been reduced in number and their activities directed toward other public health problems. In 1944 there were 29 such posts

TABLE 1

Application of insecticides in the valleys of the Province of Tarapacá, 1945–1953

Year	Number of applications	Houses treated	Average DDT per m².
1945	45	1.920	0.16
1946	42	3.188	0.57
1947	24	2.980	1.18
1948	19	1.551	2.00
1949	8	627	3.90
1950	3	459	2.60
1951	3	104	—
1952	4	253	4.70
1953	3	60	2.00

located in 8 valleys or oases. At present there are only 2, and most surveys are conducted by experts transferred for that purpose from the main Health Centers in Arica and Iquique. Any breeding places discovered are sprayed with DDT, Gammexane, or Dieldrin suspensions and emulsions. At times, when deemed advisable, minor sanitation works were carried out, such as filling stagnant pools, repairing canals, etc. Since 1951, residual house-spraying with insecticides has been confined to the area surrounding foci of anopheline breeding discovered by the surveillance service (Table 1). On some occasions, insecticides have been applied on underbrush surrounding larva foci within a radius of 50 meters for the purpose of reaching any possible zoophilic anopheline female mosquitoes.

Together with the anti-anopheline activities, an intense campaign was carried out against the *Simuliidae* which, although not vectors of disease in our country, do cause considerable annoyance to the rural inhabitants, especially in the Lluta, Azapa, and Camarones Valleys, and also cause some losses in livestock. Five general applications of insecticides were made in 1952 in the Lluta river bed, five in the Camarones River, and one in the Azapa Valley, using 193 kilograms of 50 per cent wettable DDT and 947 kilograms of 6.5 per cent Gammexane powder. These insecticides were applied to the river water every 250 meters at a ratio of 0.25 p.p.m., with satisfactory reduction of the Simuliidae population. Lately we have been applying 50 per cent wettable Dieldrin in aqueous suspension at a rate of 1.5 liters per 100 square meter of water surface.

RESULTS

Since the fall of 1948, no adult *pseudopunctipennis* have been captured in the valleys of the malaria zone in Chile. These results were accomplished mainly in three years. In 1946, only four of the ten valleys, oases and chasms of the endemic malaria zone (Fig. 1) were free from *Anopheles*; in 1947, all but two had been cleared, and in 1948 the last infested area in the Suca oasis was eliminated. Since that time, no anophelines have been discovered by intensive search throughout the entire area of the Province of Tarapacá. However, at the end of the annual summer high-water period and the consequent rise in the water table—which appears at various points as springs during the months of March to May inclu-

TABLE 2

Larval-pupal foci of A. pseudopunctipennis *in the Province of Tarapacá, 1950–1953*

	1950	1951	1952	1953
Lluta Valley	19	55	87	19
Azapa Valley	0	1	8	2
Port of Arica	0	0	0	0
Vitor Chasm	16	43	0	7

Other valleys and oases further south were negative in all years.

sive—some larval foci have been discovered in the valleys nearest Peru, such as the Lluta and Azapa Valleys. These foci generally comprise larvae in their first or second stage and only on rare occasions have *Anopheles* pupae been found. The results obtained up to December, 1953, are given in Table 2. A study of this table shows that the *Anopheles* has now been eradicated from all the valleys situated to the south of the Vitor Chasm (Fig. 1). In some of the areas, such as the Pica Oasis, *A. pseudopunctipennis* has not been found for 8 years; in others, such as the Camarones Valley, and in the oases lying to the south of that valley, no *Anopheles* has been found for the past 5 or 6 years.

DISCUSSION

Since zoological species have a tendency to reinhabit their geographic ranges, we believe it quite possible that *A. pseudopunctipennis*, which is still present in the valleys of southern Peru, may return to the Chilean valleys that are now free of them. Perhaps the fact is that the larval foci found annually in the Lluta Valley —which is the one nearest to the Peruvian valleys, although it is separated from them by a desert more than 50 kilometers long—have their origin in adult *Anopheles* transported passively on the vehicles that daily traverse the international highway, or else actively follow the bands of muleteers that travel the numerous paths between the Peruvian valleys and the central Lluta Valley. That is where the larval foci have been confirmed most frequently and in the earliest stages. In contrast, the upper part of this valley has remained free of *Anopheles* for five consecutive years (Fig. 1).

From these primary foci that appear in the Lluta Valley, some adults might actively follow the muleteers' animals as far as the Azapa Valley and from there eventually reach even the Chaca section in the lower part of the Vitor Chasm, which has the greatest traffic, since the north and south highway passes through that point for three or four kilometers (Fig. 1).

Both hypotheses are supported by several facts. On many occasions since 1925 (Massa, 1929) and later during the campaign, female *A. pseudopunctipennis* have been found in the interior of vehicles, railway cars, and automobiles; moreover, they have been seen flying around troops for several kilometers in the desert, or resting on the loads or the harness of the animals. Presumably, we are dealing mainly with zoophilic specimens of *A. pseudopunctipennis*.

The results, so far, justify the hopes expressed by the late Professor Juan Noé,

which we share, of eradicating *A. pseudopunctipennis* in all the Chilean valleys that were formerly malarious. In order to achieve this objective, surveillance of the affected sectors should be maintained and all confirmed foci should be destroyed with insecticides. In addition, the public health authorities in Peru, with whom we have concluded a border agreement, have been requested to carry on their antimalaria activities until *Anopheles* is eradicated in the Peruvian valleys adjacent to the border (Neghme, 1952). Finally, we believe that, to ensure the eradication of *Anopheles*, it is most important to achieve soil sanitation through regular and intensive cultivation of the land, a program that is fully under way in the valleys of northern Chile.

SUMMARY

Anopheles pseudopunctipennis, the only species present in formerly endemic malaria zones of Chile, can no longer be found in most of the valleys in the northern Province of Tarapacá. Following intensive larviciding and housespraying with DDT over a period of 17 years not a single adult *A. pseudopunctipennis* has been captured since the fall of 1948, and the few larval foci found have been limited to the valleys nearest Peru. However, to achieve complete eradication, control and vigilance measures will have to be maintained for a long time, and agricultural work that facilitates the elimination of breeding places for *Anopheles* should be intensified.

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STUDIES ON SCHISTOSOMIASIS

VII. Observations on some Surfactants for Dispersing Insoluble Molluscicides

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One of the problems commonly encountered in the final evaluation of an insoluble molluscicide is the conversion of the compound into a form that can be readily and uniformly applied in field plot tests. Emulsions of the toxicant in an oil are not desirable since the oil also has molluscicidal properties. The authors in a previous study (Hunter, Freytag and Ritchie, 1952a) involving insoluble molluscicides in field tests, used different surface-active agents to dissolve the compounds. The resulting concentrates were diluted with water. In many instances a concentrate could be prepared by using several different surfactants, but their stability varied markedly when added to water. To determine which of twenty-two available emulsifiers, mostly non-ionics, were best adapted for each of seven insoluble phenol derivatives, the following study was conducted.

MATERIALS AND METHODS

One tenth gm. of each molluscicide was added to 0.5 ml. of each surfactant and allowed to stand two hours; subsequently each mixture was triturated with a stirring rod and poured into sufficient water to obtain the minimum lethal dilution of the compound. These mixtures were vigorously shaken for approximately 20 seconds. Observations were recorded on the stability of the dispersion at hourly intervals for four hours and at twenty-four hours. If any appreciable sedimentation occurred during these intervals in the form of either a creamy layer, oily residue or precipitate, the degree of redispersibility was determined by shaking again.

Prior to field tests, compatibility studies were conducted as follows: 0.1 gm. of water-insoluble molluscicide in 0.5 ml. of alkyl aryl polyglycol ethers (Tergitol NPG) was added to 99.4 ml. of a 1:1000 dilution of the principal active ingredient of a soluble preparation. The final dilution of the separate molluscicides was 1:1000 or 1:500 collectively; these mixtures were subjected to the same procedure as used for single dispersions. Attempts were made to prepare emulsible concentrates containing different solvents and emulsifiers of the compounds that were insoluble in the surfactants or which yielded oily residues or precipitates. These trials were made primarily to determine whether sufficiently stable emulsions, applicable for our field tests, could be formulated without using special equipment.

The concentrate was prepared by dissolving a small amount of mollusicide

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in a solvent, adding a recorded amount of the emulsifier and adjusting the specific gravity of the solution to as near 1.0 as possible by varying the proportions of the solvent and/or emulsifier. Concentrates of specific gravity below 0.95 were difficult to convert into stable emulsions even though the emulsifier itself was suitable for the solvent being used. When incompatibility occurred, trials were made using different solvents and/or emulsifiers.

Several ml. of the prepared concentrate were added to a known amount of water, usually 100 ml., in a mixing cylinder and thoroughly agitated for one minute, care being taken to shake each mixture in the same manner and for the same length of time. The stability of the resulting emulsions was observed, with the aid of illumination from a desk lamp, at hourly intervals for four hours and then at the end of twenty-four hours. If unstable emulsions were detected during these observations, they were reshaken to determine whether the separated products, such as oil, residue or cream layer, could be redispersed. The volume of the separated products after twenty-four hours was recorded in ml. per 100 ml. of emulsion, i.e., ml. per cent.

Attempts were made to emulsify the following: 2,4,6 trichlorophenol 90% (Dowcide 2-S); 2,4 dichlorophenol; 2-chloro-6-phenylphenol; (4 (and 6) chloro-o-phenylphenol 85% (Dowcide 31); copper pentachlorophenate and pentachlorophenol. Tests were performed on three of these molluscicides to secure two or more emulsions of each, using different emulsifiers and/or solvents or the same ones in varying proportions. This was done in order to find the best solvent-emulsifier combinations for a particular molluscicide and, also, to obtain emulsions whose stability varied.

Emulsions of high molluscicidal content were sought in most instances to determine the maximum of toxicant that could be suspended with the available materials, it being assumed that if a concentrated emulsion was fairly stable a more dilute emulsion could probably be prepared by using different proportions or kinds of solvents and emulsifiers.

RESULTS

Each of the one hundred and fifty-four concentrates of a surface-active agent and molluscicide when added to water yielded, after varying intervals, a uniform dispersion without any visible evidence of separation, oily residue, creamy residue, slight precipitate or complete separation of the toxicant. All of the surfactants except three formed one or more dispersions in which a redispersible creamy residue occurred after one to twenty-four hours. Judged on the basis of the number of mixtures that were still stable after twenty-four hours, the following preparations appeared to have the best dispersing properties: alkyl aryl polyglycol ethers (Tergitol NPG), polyethylene glycol 600 or 400, an alkyl aryl polyoxyethylene derivative (Antarox A-400) and one of unknown composition (Antarox B-201). The first chemical was the most versatile, having yielded dispersions for five of the seven insoluble molluscicides, whereas each of the other preparations yielded only three. Similar suspensions of one to two compounds were obtained by each of nine surfactants.

Compatibility of sodium 2,4,5 trichlorophenate 85% (Dowcide B) with each of four insoluble molluscicides dispersed in water by alkyl aryl polyglycol ethers (Tergitol NPG) was superior to that of sodium pentachlorophenate 75% (Dowcide G) with the same compounds. The ingredients in three of the mixtures containing the former sodium compound were still in suspension after twenty-four hours, in contrast to only one mixture of similar stability containing the latter sodium compound; both preparations were highly compatible with 2,4,5 trichlorophenol.

Two satisfactory emulsions of 2,4,6 trichlorophenol (Dowcide 2-S) were obtained in dilutions of 1:140 and 1:250. The cream layers, noted after twenty-four hours but not present after four hours, were easily redispersed by a few inversions of the container. Emulsions containing a strong concentration of 2,4 dichlorophenol (1:55) were still stable after four hours when emulsified by a sulfated alkyl ester anionic (Emulside 65). The same formulation using a modified anionic hydrocarbon sulfonate (Skil 234B) as an emulsifier was less satisfactory. The chemical 2 chloro-6-phenylphenol was easily emulsified by xylene and polyoxyethylene sorbitol oleate-laurate (Atlox 1045-A). The most difficult compound to emulsify was (4 and 6) chloro-o-phenylphenol 85% (Dowcide 31); a fair emulsion, stable for only one hour, was obtained.

DISCUSSION

Dispersions of molluscicides. Paratertiary butylphenol was the most readily dispersible of the molluscicides. Although this chemical is reported as water soluble (Hodgman, 1946) the technical grade that was used did not dissolve in proportions of 0.1 gm. per 100 ml. of water. Heating or allowing the compound mixed with water to stand over night did not aid dissolving. Usually, fine flakes of the toxicant would float on the surface except for those surfactants having pronounced dispersing properties, such as alkyl aryl polyglycol ethers, polyethylene glycols and several of the alkyl aryl polyoxyethylene class.

The chemical 2,4 dichlorophenol is reported (Hodgman, 1946) to be soluble (0.46 gm. per 100 ml. of water); however, this was not evident when tested as previously described. Only one surfactant (Tergitol NPG) yielded a dispersion or possibly a combination of dispersion and solution that gave maximum stability. It is possible that water containing a particular surfactant has a greater dissolving action in some insoluble compounds This situation may have obtained in our samples of 2,4 dichlorophenol and paratertiary butylphenol.

It is interesting to note differences in dispersibility of the structurally related 2,4,5 trichlorophenol; 2,4,6 trichlorophenol 90% (Dowcide 2-S); and 2,4 dichlorophenol. Physically the compounds are quite similar. Whereas the first produced seven stable dispersions, applicable for plot tests, in addition to several satisfactory mixtures which formed redispersible cream layers in one to four hours, dispersions of the last two compounds usually produced oily residues. It is doubtful that the molluscicides were altered chemically by the surfactants which were mostly non-ionics.

The dispersion of 2 chloro-6-phenylphenol by a sulfated alkyl ester anionic

(Emulside 65) was characterized by fine glistening crystals which formed in the mixture after about one hour; these grew slightly larger and settled in small quantities after the third hour. At this time the crystals could be broken into smaller particles by shaking the container. This molluscicide gave unsatisfactory results in preliminary plot tests (Hunter, Freytag, *et al.*, 1952a).

Several effective molluscicides are known; however, the problem in control is to secure a uniform coverage of the snail habitat with the toxicant. This involves many factors such as size and adhesiveness of molluscicide particles, size of spray drops, and force or pressure needed in sprayers, etc.

It is realized that some of the surface-active agents were not designed as dispersants. However, each of these preparations except three yielded one or more dispersions that formed redispersible cream layers in one to four hours. These could have been used practically in field plot tests providing they were used in sprayers that emptied their contents before separation of the cream layer occurred. The ratio of molluscicide to emulsifier was set at a minimum (1:5) as a means of screening preparations having pronounced solubilizing and dispersing properties. A more accurate adjustment of the amount of each surfactant would have improved the stability of some of the dispersions.

Some surfactants may have lethal properties, although none in this study were lethal in practical doses. Langrange *et al.* (1950) found that the surface-active agents cetyl trimethyl ammonium bromide and dimethyl alkylbenzyl ammonium chloride were lethal at dilutions of 1:100,000 to *Australorbis glabratus* and schistosome cercariae. Hence, it is worthwhile to investigate further the use of lethal surfactants for dispersing or emulsifying insoluble molluscicides.

Mixtures of soluble and insoluble molluscicides. Differences in composition and amount of the inert portion of the two soluble molluscicides sodium pentachlorophenate 75% (Dowcide-G) and sodium trichlorophenate 85% (Dowcide-B) may have contributed, partly, to differences in compatibility of the mixtures of each of these chemicals and the same insoluble compounds. Evidence of a residual effect by the soluble molluscicide, sodium pentachlorophenate (Santobrite), has been noted (Hunter, Freytag *et al.*, 1952b); however, it was assumed that this action could be prolonged, or killing of snails speeded, by the addition of an insoluble toxicant to a solution of this preparation. A few of these mixtures killed snails faster than either of the compounds used alone in an equal dosage (Hunter, Freytag *et al.*, 1952a). *Oncomelania nosophora* in habitats inaccessible to sprays applied by current control methods or in dry areas where it is protected by its firmly retracted operculum may survive present control measures. A molluscicide having pronounced residual properties is needed for application in such habitats.

In supplementary tests copper sulfate solutions, 1:1000, were compatible with an equal volume and dilution of 2,4,5 trichlorophenol dispersed by three parts instead of the usual five parts of alkyl aryl polyglycol ethers (Tergitol NPG). These mixtures formed creamy residues after three hours which were still redispersible and apparently unchanged after standing two months.

Emulsions of molluscicides. These molluscicidal emulsions must be regarded

as preliminary in nature since the perfecting of an emulsion system often involves special equipment and a suitable choice from a variety of solvents and of surface-active agents While the emulsions prepared were satisfactory for evaluation in field tests, time only permitted the testing of one (Hunter, Freytag *et al.*, 1952a).

Concentrated emulsions of DDT yield a more adhesive or weathering resistant residue on foliage than when applied as a suspension (Keiser and Henderson, 1951). Possibilities of similar results with molluscicides warrant investigation for increasing their efficiency especially in the control of amphibious snails.

The chemical (4 (and 6) chloro-o-phenylphenol 85% (Dowcide 31), a brown, viscous, syrupy liquid, was difficult to emulsify. An isomer of one of the ingredients in this preparation, 2-chloro-6-phenylphenol, a white crystalline solid, emulsified readily. The compound possessed residual properties in the laboratory as a viscous adhesive film was formed when 2 or 3 ml. of a 1:250 dilution in ether was placed on filter paper, which was lethal to snails for several days. Large doses as an emulsion gave good results in plot tests (Hunter, Freytag *et al.*, 1952a). An improved emulsion would probably require smaller dosages to effect the same degree of control.

Pentachlorophenol is an effective molluscicide (McMullen *et al.*, 1951) and may interfere with the reproduction of certain aquatic snails in Egypt (Halawani *et al.*, 1951). More effective results and possibilities of a residual action may be obtained with this compound in emulsion form. The effect of pentachlorophenol emulsions on *O nosophora* is not known. This chemical was not soluble in the available surfactants and only slightly soluble in the common solvents. The emulsion using propylene glycol ethyl ether (Dowanol 34B) although stable for only one hour, was still redispersible after standing two months. Results with propylene glycol methyl ether (Dowanol 33B) were less satisfactory.

Copper pentachlorophenate also has a limited solubility in the common solvents, and yielded concentrates containing only 8 per cent of the toxicant. Unsatisfactory results were obtained when attempts were made to disperse it with either Carbowax 1500 or 4000. Copper pentachlorophenate dissolved in kerosene saturated with isoprophylphenol and emulsified with a tertiary dodecyl polyglycol thioether (Sharples 2543) gave satisfactory emulsions when diluted 1:2000. This is probably too dilute a concentration for the amphibious snail *O. nosophora* but may be satisfactory for aquatic snails.

A review of the literature reveals that besides the soluble sodium pentachlorophenate, highly effective against both aquatic and amphibious snails (Berry *et al.*, 1950; McMullen *et al.*, 1951; Hunter, Freytag *et al.*, 1952b), nearly all other effective molluscicides are insoluble, the best of these being (DN-1) dinitro-o-cyclohexylphenol 40% (Kuntz and Wells, 1951; McMullen *et al.*, 1951). The value of using surfactants in such cases is evident.

SUMMARY

A rapid method for screening surfactants adapted for dispersing particular molluscicides is outlined. The characteristic behavior of surface-active agents is

illustrated in a series of tests involving twenty-two well-known surfactants and seven insoluble molluscicides which were chlorinated phenol derivatives. Efficient dispersants screened in this study together with comments on their dispersibility are presented.

Potential possibilities in the use of soluble and insoluble molluscicides and simple methods of testing their compatibility are described, using two effective soluble molluscicides and four insoluble compounds. A simple method is given for preparing molluscicidal emulsions which is satisfactory for field plot tests. Characteristics of eleven emulsions of six different molluscicides are recorded.

ACKNOWLEDGEMENTS

Grateful appreciation is extended to the companies listed below for their generous cooperations in furnishing chemicals.

Union Carbide and Carbon Corporation; Antara Products, General Dyestuff Corporation; Atlas Powder Company; E. F. Drew and Company Incorporated; Rohm and Haas; Griffin Chemical Company; Emusol Corporation; Glyco Products Company Incorporated; Van Dyke and Company; Gallowhur Chemical Corporation; Monsanto Chemical Company; Sharples Chemicals Incorporated; Dow Chemical Company.

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BREEDING MEDIA OF COMMON FLIES

II. In Rural Areas

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Because the housefly, *Musca domestica*, is involved in transmission of diarrheal diseases (Watt and Lindsay, 1948; Lindsay *et al.*, 1953) and constitutes health hazards of other types, considerable effort has been expended to obtain information that will serve as a basis for development of effective control measures. Recent studies to determine the types and locations of breeding media which produce the more common muscoid flies in urban areas (Haines, 1953) show that the housefly utilizes a greater variety of naturally occurring refuse than do other common species. The present report concerns results of investigations on the breeding habits of flies in small communities in southwest Georgia.

PLAN OF STUDY

The study was made from May, 1952, through April, 1953, in three rural communities, each with a population of approximately 1,000. The towns were about fifteen miles apart and are typical southern truck-crop centers. Animal-exclusion ordinances were not enforced in any of the towns; cows, hogs, and chickens were kept by numerous householders throughout the year. Privies were used by most of the inhabitants in each town; less than one-half of the dwellings had access to sewage disposal systems. A centrally-located drilled well in each town supplied water to about one-half of the population and privately drilled wells furnished the remainder. Produce shipping centers for watermelons, cantaloups, okra, beans, and other truck crops were the only industrial areas of importance in producing flies. They were present in each town. Garbage pickup was scheduled twice weekly during the period of study. Schedules were maintained except for annual increases when spillage and decomposition of the waste from produce centers caused major sanitation problems.

Observations were made to detect occurrence of two different breeding materials each day in each of the three towns, five days per week when weather permitted. Approximately equal periods of time were spent in locating and examining each medium for larval infestation.

Twenty kinds of breeding material were surveyed for degree of infestation (Table 4) and relative abundance. When examination revealed the presence of larvae, samples were collected individually in one-fourth pint capacity ice cream cartons equipped with friction lids. If fewer than nine samples were found, all of each kind of breeding material was brought to the laboratory. If more than nine of each kind of breeding material was present, nine of the most heavily in-

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fested samples from the total of eighteen possible samples of each material were brought to the laboratory in accordance with the routine sampling schedule.

At the laboratory, the lid was removed from quarter-pint container and each sample (in its container) was placed in a one gallon ice cream carton upon a one-inch layer of moist sand that had been washed and sterilized previously. This procedure provided for pupation of the larvae which migrated from the breeding material. The central cardboard disks in the lid of the one gallon carton were replaced with a 24-mesh screen wire disk to permit ventilation. The rearing was done in an insectary maintained at a fairly uniform temperature (mean of 81°F., range 76° to 85°) and relative humidity (mean of 74 per cent, range 69 to 81 per cent). Daily checks were made on each lot at approximately the same time each day. Adult flies which emerged were removed from the rearing container, killed, and stored for later identification.

TABLE 1

The occurrence, larval infestation and adult production from five principal categories of fly breeding media found in three rural towns in southwest Georgia, May, 1952, through April, 1953

Breeding Material Categories	Number of Occurrences	Number Infested with Larvae	Per cent Infested with Larvae	Average Number of Adults Reared per Sample
Animal pen litter*	1781	1183	66.4	31.4
Animal wastes†	633	124	19.6	108.1
Animal excrements‡	1986	1120	56.4	58.3
Fruit-vegetable wastes§	309	86	27.8	57.0
Miscellaneous wastes¶	705	313	44.4	32.1

* Animal pen litter refers to a mixture of domestic animal excrement, straw, hay, spilled grain and urine.

† Animal wastes included: (1) bones of cows and hogs, whether cooked or uncooked; (2) carcasses of small animals; (3) entrails found near garbage cans; and (4) fish remains.

‡ The various animal excrements, including human excrements, were collections of stools or individual droppings and privy contents.

§ Fruit and vegetable wastes included fruits and/or vegetables, in the field or harvested, cooked or uncooked.

Miscellaneous media consisted of: (1) garbage in and around a container, located near an occupied dwelling; and (2) soil, which included wash and/or dishwater and worm beds.

TABLE 2

Production of Musca domestica *from five principal fly breeding media categories, 1952–53*

Breeding material Categories	Number of Occurrences	Number Infested with *Musca domestica*	Per cent Infested with *Musca domestica*	Average Number of *Musca domestica* per Sample
Animal pen litter	1781	569	31.9	32.5
Animal wastes	633	5	0.8	6.4
Animal excrements	1986	158	8.0	60.2
Fruit-vegetable wastes	309	26	8.4	55.5
Miscellaneous wastes	705	122	17.3	20.6

TABLE 3

The productivity of all common fly species (other than Musca domestica *from five principal fly breeding media categories, 1952–53*

Species	Breeding Material Categories	Number of Occurrences	Number of Infestations	Per cent Infested	Average Number of Flies per Sample
Stomoxys calcitrans	Animal pen litter	1781	220	12.4	6.8
	Animal wastes	633	0	0.0	0.0
	Animal excrements	1986	47	2.4	7.9
	Fruit-vegetable wastes	309	5	1.6	1.4
	Miscellaneous wastes	705	5	0.7	3.8
Phaenicia pallescens	Animal pen litter	1781	4	0.2	4.0
	Animal wastes	633	34	5.4	145.8
	Animal excrements	1986	2	0.1	9.5
	Fruit-vegetable wastes	309	4	1.3	1.0
	Miscellaneous wastes	705	23	3.3	51.3
Phaenicia sericata	Animal pen litter	1781	6	0.3	25.3
	Animal wastes	633	33	5.2	74.4
	Animal excrements	1986	59	3.0	23.6
	Fruit-vegetable wastes	309	0	0.0	0.0
	Miscellaneous wastes	705	6	0.9	89.7
*Sarcophagula occidua**	Animal pen litter	1781	3	0.2	4.0
	Animal wastes	633	0	0.0	0.0
	Animal excrements	1986	10	0.5	11.6
	Fruit-vegetable wastes	309	0	0.0	0.0
	Miscellaneous wastes	705	0	0.0	0.0
Sarcophaga spp.	Animal pen litter	1781	83	4.7	7.8
	Animal wastes	633	37	5.8	31.7
	Animal excrements	1986	535	26.9	32.8
	Fruit-vegetable wastes	309	6	1.9	5.3
	Miscellaneous wastes	705	9	1.3	5.1
Muscina spp.	Animal pen litter	1781	23	1.3	9.9
	Animal wastes	633	3	0.5	1.3
	Animal excrements	1986	17	0.9	12.1
	Fruit-vegetable wastes	309	8	2.6	18.4
	Miscellaneous wastes	705	6	0.9	7.3
Ophyra leucostoma	Animal pen litter	1781	43	2.4	13.8
	Animal wastes	633	0	0.0	0.0
	Animal excrements	1986	61	3.1	22.7
	Fruit-vegetable wastes	309	8	2.6	4.5
	Miscellaneous wastes	705	8	1.1	10.4
Hydrotaea spp.	Animal pen litter	1781	9	0.5	8.1
	Animal wastes	633	1	0.2	1.0
	Animal excrements	1986	18	0.9	8.9
	Fruit-vegetable wastes	309	3	1.0	6.0
	Miscellaneous wastes	705	0	0.0	0.0

TABLE 3—*Continued*

Species	Breeding Material Categories	Number of Occurrences	Number of Infestations	Per cent Infested	Average Number of Flies per Sample
Callitroga macellaria	Animal pen litter	1781	1	0.1	2.0
	Animal wastes	633	5	0.8	101.6
	Animal excrements	1986	0	0.0	0.0
	Fruit-vegetable wastes	309	2	0.6	2.5
	Miscellaneous wastes	705	2	0.3	76.5
Limnophora spp.	Animal pen litter	1781	18	1.0	2.2
	Animal wastes	633	0	0.0	0.0
	Animal excrements	1986	76	3.8	2.3
	Fruit-vegetable wastes	309	0	0.0	0.0
	Miscellaneous wastes	705	7	1.0	2.3
Calliphora spp.	Animal pen litter	1781	1	0.1	3.0
	Animal wastes	633	6	0.9	131.2
	Animal excrements	1986	0	0.0	0.0
	Fruit-vegetable wastes	309	2	0.6	4.5
	Miscellaneous wastes	705	1	0.1	1.0
Phormia regina	Animal pen litter	1781	0	0.0	0.0
	Animal wastes	633	4	0.6	43.3
	Animal excrements	1986	1	0.1	1.0
	Fruit-vegetable wastes	309	0	0.0	0.0
	Miscellaneous wastes	705	0	0.0	0.0

* Although climatic conditions were extremely favorable for the production of *S. occidua* during the 1951–53 seasons, the distribution of this major excrement breeder in the area remained far below the 1949–50 level. This slow build-up in the population of the species was attributed to an abnormally cold winter in 1950 and to the proximity of its northern limits of distribution.

RESULTS

Animal pen litter and animal excrement occurred more frequently and were found to support more fly breeding than all other main categories of breeding material (Tables 1, 2, 3, and 4). In contrast to conditions found in larger communities (Haines, 1953), packing plant and paunch manure were of minor importance. There was a high rate of breeding in slop-soaked soil caused by the common practice of disposing of washwater and dishwater on the soil adjacent to the rear entrances of homes, and in garbage. Frequent intervals of dry weather during the year made slop-soaked soils highly productive of flies.

The total production of houseflies from animal refuse (animal pen litter, animal excrements, and animal wastes) was similar (89 and 87 per cent, respectively) in both urban (Haines, 1953) and rural areas.

A comparison of the distribution of the species of muscoid flies in terms of the numbers of infestations showed that none approached that of *M. domestica* as shown in Tables 2 and 3. This finding compared quite closely with results of a

TABLE 4

The productivity of house flies from five chief categories of breeding materials, 1952–53

Breeding Material Categories	Breeding Material Items Comprising Each Category	Number of Occurrences of Items	Number of Samples Infested with House Flies	Per cent Observed Occurrences Infested	Average Number of House Flies Infested per Sample
Animal pen litter	Fowl	352	83	23.6	49.9
	Pig	353	135	38.2	53.6
	Cow	324	95	29.3	13.0
	Horse	337	134	39.8	26.2
	Cow and horse mixed	268	104	38.8	18.4
	Rabbit	59	1	1.7	1.0
	Goat	88	17	19.3	19.4
Animal wastes	Bones	304	1	0.3	4.0
	Carcasses	116	2	1.7	11.0
	Entrails	52	2	3.8	3.0
	Fish	161	0	0.0	0.0
Animal excrements	Human	325	20	6.2	63.9
	Dog	348	7	2.0	56.4
	Hog	349	33	9.5	101.1
	Cows	337	10	3.0	19.2
	Horse	338	60	17.8	36.8
	Privy	289	28	9.7	75.1
Fruit-vegetable waste	Fruit and/or vegetables, in field or harvested, cooked or uncooked	309	26	8.4	55.5
Miscellaneous wastes	Garbage	352	39	11.1	16.4
	Slop soaked soils	353	92	26.1	22.0

TABLE 5

The seasonal production of the more common flies from all items of breeding media, 1952–53

Fly Species	Numbers Reared			
	Spring	Summer	Fall	Winter
Musca domestica	11522	13177	5561	1713
Stomoxys calcitrans	1134	138	442	175
Sarcophagula occidua	6	7	106	9
Phaenicia spp.	5607	3257	3386	229
Phormia regina	174	0	0	0
Callitroga macellaria	489	162	17	0
Sarcophaga spp.	6145	5429	5962	1914
Others*	2542	155	437	1112
Total	27619	22325	15911	5152

* Others include: *Muscina* spp., *Ophyra leucostoma*, *Hydrotaea* spp., *Limnophora* spp., and *Calliphora* spp.

similar study in urban areas. The occasional high production of certain other species from certain breeding materials is offset by its relatively infrequent occurrence or by being infested only by one or two species of flies such as *Calliphora* spp., *Phormia regina* and *Callitroga macellaria*.

Nineteen of twenty breeding materials tested during the course of the study produced houseflies (Table 4). No other species bred in so many. Animal pen litter which contained large amounts of digested roughage (hay, straw and corn husks) was found to exceed all others in the number of samples infested with houseflies, as well as in the number of house flies produced per infested sample.

SUMMARY

A study of sources and seasonal abundance of the more common muscoid flies in rural areas from May, 1952, through April, 1953, showed that *Musca domestica* was produced by nineteen of twenty breeding materials throughout the period of the study. No other species approached the housefly in this respect. Two chief categories of animal refuse (excrements and animal pen litter) occurred most frequently in media examined in the towns and were found to contribute 87.1 per cent of all houseflies produced from all types of breeding material. Miscellaneous, animal, and fruit-vegetable wastes were found to occur less frequently and to produce fewer flies. The abundance of *Phaenicia* spp. (garbage flies) and *Sarcophaga* spp., while less than houseflies, was markedly uniform throughout the year and is suggestive of the relatively minor importance of environmental factors. These factors are however adverse to houseflies.

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STUDIES OF DISTRIBUTION AND HABITAT OF COCKROACHES IN SOUTHWESTERN GEORGIA, 1952–53

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The close association of many common species of cockroaches with human beings has fostered much interest regarding their importance as disseminators of certain enteric and other diseases of man. Experimental work has been done to ascertain the viability and survival in them of several enteric pathogens using the more common species of these insects as experimental hosts (Mackerras and Pope, 1948; Wedberg *et al.*, 1949; Olsen and Rueger, 1950; Janssen and Wedberg, 1952; Jung and Shaffer, 1952). Simultaneously and immediately succeeding these investigations, natural infection with several pathogenic organisms in cockroaches was demonstrated. (Mackerras and Mackerras, 1949; Bitter and Williams, 1949; Syverton *et al.*, 1952; Eads *et al.*, 1954.) Three studies were made in Texas and Arizona to determine the range of dispersion of sewer-infesting roaches and the extent to which they might carry disease pathogens from sewerage systems to human habitations (Schoof and Siverly, 1954; Eads *et al.*, 1954; Jackson and Maier, 1955). All three investigations showed at least sporadic dispersal of one species (*Periplaneta americana*) between sewers and human habitations.

The study here reported was made in twelve towns in southwestern Georgia from August, 1952, through July, 1953, in an area where the bulk of the inhabitants do not have access to sewerage systems and, instead, use privies as a means of excreta disposal.

PLAN OF STUDY

A routine roach-trapping schedule of harborages inside homes, outside homes, and in or around privies in each of twelve towns was begun in August, 1952. The sampling was planned to permit a comparison of the relative seasonal abundance of each of the more common species in the various habitats. Collections were made by the use of specially constructed traps which were baited with dry commercial fox food of the kind used by Olson[3]. Approximately 80 trappings inside homes, outside homes, and in or around privies were made and recorded each week in three towns when weather permitted. In this manner, the various habitats in all twelve towns were trapped (for roaches) each month from August, 1952, through July, 1953. The number of trappings exceeded 100 per week when sewer manholes were included in the program in January, 1953. All baited traps remained at each trap site for 48 hours, after which they were returned to the laboratory and placed in deep freeze prior to identification of adult species.

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[3] Olson, T. A., 1951. Personal communication.

TABLE 1

Comparison of distribution (by season) of cockroaches trapped in four environments in southwestern Georgia, 1952–1953

Environ Trapped	Season	No. of Traps Set	No. of Premises Trapped	*Periplaneta fuliginosa*		*P. americana*		*P. brunnea*		*Blattella germanica*	
				Av. no. adults per trap	% premises producing	Av. no. adults per trap	% premises producing	Av. no. adults per trap	% premises producing	Av. no. adults per trap	% premises producing
Inside homes	Spring	1186	244	0.07	15.9	0.02	6.1	0.09	8.6	1.71	34.8
	Summer	1110	266	0.15	19.2	0.09	10.1	1.54	13.9	0.47	23.7
	Fall	502	136	0.45	25.7	0.01	0.7	0.07	5.9	6.57	48.8
	Winter	915	195	0.04	9.7	0.01	1.5	0.01	10.8	2.03	33.8
	Total	3713	841								
Outside homes	Spring	592	135	0.10	15.5	0.01	0.1	0.01	1.5	0.05	8.1
	Summer	105	54	0.34	11.1	0	0	0.11	5.5	0	0
	Fall	96	41	0.27	12.2	0	0	0.07	2.4	0.17	9.7
	Winter	234	87	0.21	10.3	0	0	0.05	2.3	0.01	5.7
	Total	1027	317								
In or around privies	Spring	43	20	0.11	10.0	0.02	5.0	0	0	0.07	10.0
	Summer	46	16	1.21	37.5	0.83	31.2	0.25	6.2	0.04	0.6
	Fall	44	20	0.77	30.0	0	0	0.5	5.0	0.02	5.0
	Winter	31	23	1.20	26.1	0	0	0	0	0.06	4.3
	Total	164	79								
Sewer manholes	Spring	772	221	0.07	5.5	7.2	88.3	0	0	0	0
	Summer	735	246	0.01	1.6	5.1	89.3	0.03	0.7	0.01	1.1
	Winter	564	157	0.01	0.7	0.9	23.3	0.01	2.7	0.01	3.9
	Total	2071	624								

RESULTS

German roaches, *Blattella germanica*, occurred in greater abundance inside homes than all other species trapped (Table 1). This species was less abundant and *P. americana* and *P. brunnea* more abundant during the hottest months of the year than during colder parts of the year.

Other species collected inside homes but not included in the tabular presentation were two brown-banded *Supella supellectilium* and four Surinam roaches, *Pycnoscelus surinamensis*. The former were trapped in the kitchen of one home in which no other species were collected, and the latter were individually trapped during August, 1952, in four separate homes located in four different towns. All specimens of both species were female.

P. fuliginosa consistently outnumbered all other common species of roaches trapped outside homes. Only one *P. americana* was trapped outside homes but significantly greater numbers were trapped inside homes, privies, and sewer manholes. These results strongly suggest that *P. americana* either flies directly to and from its more favorable environment or that possible migration occurs between sewers and homes through plumbing traps as suggested by previous investigators (Eads *et al.*, 1954; Schoof and Siverly, 1954; Jackson and Maier, 1955).

P. fuliginosa generally was more commonly caught in and around privies than were the remaining species, but during the summer months the numbers of *P. americana* trapped and the numbers of privies producing them approached those of *P. fuliginosa*. *P. brunnea* was taken in and around privies only during the warmer months of the year.

A comparison of the distribution of all common roaches in sewer manholes during a three season period is shown in Table 1. A gradual increase in the number caught, presumably of the population of *P. americana*, was evidenced from March through June, 1953, both in numbers of adults trapped per month and in the per cent of manholes producing the species. Heaviest populations of *P. americana* in June were followed by a gradual decline until the end of the study in September, 1953. During the spring and summer seasons, they were most numerous in sewer manholes; also during the same period, the largest numbers of this species occurred inside homes and in or around privies.

Although trap collections in and around privies were comparatively small, *P. fuliginosa* was the predominant species frequenting this environment, both in numbers of adults captured and in numbers of premises producing this species. The German roach, *B. germanica*, occurred in greatest abundance inside homes as compared with all other species and, while few in numbers, was rather evenly distributed outside homes and in and around privies. Although *P. fuliginosa*, *P. brunnea*, and *B. germanica* were trapped in small numbers in sewer manholes during the period of study, 98.9 per cent of the total adult collections were American roaches, *P. americana*.

SUMMARY

A study of distribution and habitat of the common cockroaches from August, 1952, through September, 1953, showed that *B. germanica* outnumbered all other

common species (86.6 per cent) inside homes and that *P. americana* was the most prevalent (99 per cent) in sewer manholes. The distribution of *P. americana* suggests a direct migration from more favorable to less favorable environments and suggests that population pressure of the species in sewerage systems may be a factor inducing movement to homes. *Blattella germanica* was less abundant and *P. americana* and *P. brunnea* more abundant during the late spring and summer months.

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COMPLETE AND SPONTANEOUS RECOVERY FROM THE BITE OF A BLUE KRAIT SNAKE (*BUNGARUS CAERULEUS*)

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The blue krait is one of the most dreaded of the Oriental poisonous snakes. There are approximately six known species belonging to the genus *Bungarus*; they are found throughout Southeastern Asia and the Malay Archipelago (Ditmars, 1933) and are more common than the cobras in heavily populated areas. Physically they vary from four to six feet in length and possess smooth, shiny scales, varying in color from dark brown to bluish black, with yellow cross bands. The blue krait is alleged to cause a terrible loss of human life and in our search of the literature, only one case of recovery from its bite was found (D'Afreu, 1939). This snake's venom rates second in toxicity to that of the tiger snake of Australia, the latter being the most poisonous snake in the world. In estimating venom toxicity, the Indian cobra is used as a standard of measure. The krait is considered 15 times, and the tiger snake 25 times more toxic than the cobra (Shattuck, 1954). The venom is a neurotoxin, and in most cases individuals bitten die from respiratory failure. Death usually ensues in a matter of hours after the bite, but according to Amaral (1925) some individuals may live as long as six or more days. The demise is usually preceded by general depression, anorexia, muscular weakness, rapid emaciation, oliguria and other symptoms of slow intoxication. In an incident presented by Shattuck (1954), two men were sleeping on the ground when a blue krait bit one on the index finger and immediately afterward bit the other man on the knee. In neither case were there visible local symptoms except two fang marks. Paralysis of the vocal cords ensued so rapidly that the first man was only able to utter three words and the second man could not speak at all. Neither was able to rise from the ground and in both cases death ensued from respiratory failure. The individual bitten on the index finger died 19 hours later, while the one bitten on the knee died half an hour later.

CASE REPORT

This is the case of one of us (W. E. H.), a white male, age 43, who is the director of a serpentarium in Miami, Florida. At this establishment, venoms are prepared for the production of pharmaceutical products and for basic venom research. Since September 10, 1948, this individual has been engaged in a project of self-immunization. The venom from the African Cape cobra (*Naja flava*) was used as the antigen in progressive doses. The patient received a ten microgram intradermal injection originally, and by March 1, 1951, he was receiving 40 mgm. per injection. The time interval between inoculations was progressively longer. It is interesting to note that during the interval from March, 1949, to March, 1951, this patient received several accidental bites from species related and un-

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related to the Cape cobra. He not only received bites from this snake but also from the Egyptian cobra, Indian cobra, the Florida diamondback rattlesnake, and the highly poisonous Mexican moccasin (Cantil). No serum or other specific therapy was given for any of the above bites other than that following the Cantil bite, when North American antivenin was administered plus routine supportive therapy. In November, 1953, the patient continued with his immunizations using mixed venoms. This material was obtained from the African Cape cobra, Indian cobra, and the King cobra. His last inoculation, just prior to the blue krait bite, consisted of 3 mgm. of toxin from each snake mentioned above or a total of 0.9 cc. of venom.

On March 29, 1954, at 4 p.m. the patient was attempting to extract venom from the blue krait when he received a bite on the dorsal surface of the right hand. He states that one fang entered a vein while the other embedded itself in the soft tissues. No venom had been obtained prior to the bite and the last extraction and forced feeding took place one week previously. This man continued with his extractions and feedings doing nothing about the bite, and complained of no immediate discomfort. Approximately one half hour later he manifested slight euphoria. He continued to work until 6 p.m. Soon after the accident the patient began keeping notes of his sensations and observations; the following is an account as it was recorded by him and later by his wife.

5:30 p.m.—Arm is sore all the way to axilla. Axillary lymph nodes enlarged.
6:30 p.m.—My throat is irritated and feels sore.
7:30 p.m.—Have an appointment but do not feel like going. (Patient kept this appointment, driving approximately 15 miles each way, alone).
8:00 p.m.—Unsteady on feet—arm very sore but no swelling. My jaw muscles hurt, it is becoming difficult to swallow. Pins and needles sensation in tongue. Vision beginning to blur. Chest and stomach cramps quite severe. (Patient noticed the above as he got out of his car to keep his appointment).
9:00 p.m.—Much more difficult to walk straight—I walk as if I were drunk. Chest and stomach cramps very severe. (When driving home patient had severe ptosis of eyelids). Can't keep eyes open. Must use only one eye in order to see correctly. Every muscle in my body aches. Pins and needles sensation on soles of feet. Throat very sore, teeth hurt, back of tongue is numb. My speech and thought processes do not seem to be affected.
9:45 p.m.—Arrived home—it is very difficult to walk—feel nauseated. (Patient vomited a few moments later).
10:30 p.m.—Can't breathe through nose—seems to be stuffed.
11:00 p.m.—Chills
11:15 p.m.—Can't swallow saliva—spitting into bucket—breathing through mouth.
11:30 p.m.—Radio and other sounds are tremendously magnified. Colors of the curtains seem more brilliant.
12:01 a.m.—Walked to bathroom—still staggering.
3:00 a.m.—(Notes taken by wife). He seems completely "fatigue." He can't swallow and still has all the symptoms he described. His hands and feet are extremely cold.
8:00 a.m.—Patient taken to hospital.

COURSE IN HOSPITAL

Patient entered the hospital on March 30, 1954, with all the signs and symptoms noted in his "diary." Physical examination was essentially negative except

for the neurologic manifestations as described. Temperature, pulse, respiration were all within normal limits. His blood pressure remained around 120/70. Laboratory tests consisting of urinalysis and complete blood counts were normal. For approximately 36 hours after hospitalization the neurologic abnormalities increased in intensity as did the generalized muscle pain and spasm. Nutrition consisted of intravenous glucose during the period when he could not swallow, and hot packs were applied to his spastic muscles. After the third hospital day he felt improved and wished to go home. Patient was discharged, contrary to the wish of the attending physician, on April 4, 1954. The day following his discharge he resumed his work of extracting venoms and force feeding his snakes—the cobra and the blue krait.

SUMMARY

A case is presented of a 43 year old white male who was bitten by the deadly snake, *Bungarus caeruleus*, and who made a complete recovery without specific therapy. The recovery of this individual presents the possibility of a cross protection existing between the venoms of the species *Bungarus* and those of the Indian, African and Egyptian cobras. The phenomenon of cross protection should be investigated further with the possibility of producing a common antivenin. This would not only be of great value to the Armed Forces but to the ever increasing number of individuals who travel to areas where poisonous reptiles abound.

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BOOK REVIEWS

Die Brucellose als Anthropo-Zoonose. Febris Undulans. Eine Zusammenfassende Darstellung für Ärzte und Tierärzte, By W. Löffler, D. L. Moroni and W. Frei. 193 pp., illustrated. Berlin: Springer-Verlag, 1955.

At last, in an excellent monograph, the enormous amount of clinical observations and research on brucellosis during the past 60 years is presented, and presented in an unbiased manner. The consummation of this literary enterprise was favored by the close cooperation between outstanding medical clinicians and a pathologist in veterinary medicine. The source of the human infection, correctly designated an anthropozoonosis, not a zoonosis, is admirably documented. Every aspect of brucellosis in animals is considered and fully documented. The chapter dealing with the bacteriology and serology of the *Brucella* contains a useful discussion of the blocking and the Brucella-Coombs tests and their diagnostic value. The epidemiologic, clinical and pathologic aspects of human brucellosis are masterful considerations of the present-day knowledge. Under therapy, in the discussion of the use of insoluble amphotheric oxytetracycline as recommended by Castañeda, the thought is expressed that with this antibiotic the treatment of large animals may be economically feasible. The reference list covering 23 pages is nearly complete. The symposium "Brucellosis," published by the American Association for the Advancement of Science in 1950, is omitted. Apparently publications of this type have a limited circulation in Europe. An excellent index of the authors consulted and the subject matter makes the monograph invaluable for quick reference. This enthusiastic evaluation of an important contribution to medicine and its teaching is attenuated by the notice that the copyright now includes photomechanical reproduction of portions of the monograph. This restriction is not in the interest of free exchange of knowledge!

K. F. Meyer

Manual of Medical Helminthology, by C. J. HACKETT, M.D., F.R.C.P., lately Director Wellcome Museum of Medical Science, London, with the cooperation of Professor J. J. C. BUCKLEY, D.Sc., Dept. of Parasitology, London School of Hygiene and Tropical Medicine; and the late Professor F. MURGATROYD, M.D., F.R.C.P., Dept. of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine. 330 pages illustrated. London, England: Cassell and Company, Ltd. 1954. Price 18/6 net.

This handbook of small octavo size has been prepared for physicians in the tropics needing brief authoritative information on basic and applied aspects of helminthic diseases. It is an outgrowth of requests for published material similar to that accompanying helminthic exhibits in the Wellcome Museum of Medical Science, London. The authors are eminently qualified for the task which they undertook.

The first section provides orientation on the helminths of man as a group, including definition, historical, etiologic, morphologic, life cycle, epidemiologic and pathologic aspects of the Trematoda, Cestoda and Nematoda. This is followed by a consideration of the clinical manifestations of the helminthiases, their diagnosis, treatment, prognosis and prevention.

Individual species of trematodes, cestodes and nematodes of medical importance are then taken up in orderly sequence, with especial attention to their geographical distribution, intermediate hosts (when these are required), recognition features of the agent and of the disease produced, treatment and prognosis. The text is illustrated with diagrammatic drawings of important diagnostic characters of adult worms, eggs, embryos, larvas, and molluscan intermediate hosts for the trematodes. Following the presentation of material on specific infections, there is a short list of "some helminthological terms with their derivations," although this does not always provide adequate definitions. In addition there is a "family tree" type of chart for each of the three major groups of helminths, indicating the characteristics of the successive taxonomic subdivisions of the group to which the genera and species belong. A subject index concludes the volume.

There is no bibliography although on page 1 authors are mentioned in connection with historical landmarks and other names are occasionally referred to in the body of the text. On page vi it is advised that detailed information may be obtained in *Tropical Diseases Bulletin*, or in the following texts: Faust's *Human Helminthology* (1949), Ash and Spitz's *Pathology of Tropical Diseases, An Atlas* (1945), and Belding's *Textbook of Clinical Parasitology* (1952).

This volume is what a manual should be, namely "a small book that helps its readers to understand." With relatively few exceptions the subject is accurately presented, the information is up-to-date, and most of the illustrations are helpful. Several typographical errors have been noted. The format is pleasing and the type is clear. This volume will be very helpful to physicians working in warm climates.

ERNEST CARROLL FAUST

Some Physiological Aspects and Consequences of Parasitism, by WILLIAM H. COLE, editor with 6 contributors. 90 pp., illustrated. New Brunswick, N. J.: Rutgers University Press, 1955, $2.00.

This little book contains six papers presented at the eleventh annual conference on protein metabolism of the Bureau of Biological Research at Rutgers University. There is a foreword by Cole and introductory remarks by Leslie A. Stauber calling attention to the growing interest in problems of the interactions of hosts and parasites, and of their mutual physiological modifications.

In the first paper William Trager discusses "Studies on the Cultivation of Malaria Parasites." It has been possible to support development of *Plasmodium lophurae* extracellularly for several days and to study some of its nutritional requirements independent of the host. In the second paper James W. Moulder discusses "The Protein Metabolism of Intracellular Parasites," and compares the metabolism of bacteriophages, rickettsias and malaria parasites, with special reference to the relative metabolic contributions of host

and parasite. The bacteriophage contributes no energy-producing or synthetic enzymes, only a genetic apparatus for directing the activities of host enzymes, whereas the malaria parasites use their own enzymes to appropriate the host cell's protein for their own protein synthesis. The rickettsias occupy an intermediate position.

In the third paper Clark P. Read discusses "Intestinal Physiology and the Host-Parasite Relationship." Most of the data are derived from studies on the tapeworm, *Hymenolepis diminuta*, and include studies on the quality of dietary carbohydrate and its availability to a lumen parasite, the relation of intestinal secretions to tapeworm excystation, and the interesting demonstration of a highly complex microstructure of the tapeworm surface presumably facilitating absorption. The fourth paper, by Ernest Bueding, is on "Studies of the Glycolytic Enzymes of *Schistosoma mansoni*." In this are revealed differences in the nature of enzymes catalyzing the same reactions in the parasite and in its host. It is pointed out that comparative biochemistry eventually may contribute to a more rational approach to chemotherapy. The fifth paper is on "Studies in Antibody Formation," by W. H. Taliaferro. In this he discusses antibodies from the standpoints of characterization, rise and fall in serum, organ sites of formation, anamnesis, and synthesis. Results indicate that the induction period in synthesis is a period in which synthesizing enzymes are produced, rather than a period during which amino acid-containing precursors are formed. In the sixth paper Dr. L. A. Stauber discusses "Leishmaniasis in the Hamster." The number curves of the parasites in the host and the effects of the infection on the host suggest that death results from the cumulative toxic or depletive effects of large numbers of parasites.

The subject matters of these six papers are unrelated except within the broad limits of interrelations of hosts and parasites, with special reference to their metabolism. This presentation of findings in selected areas in which further development may be expected to occur should, as hoped by the conference, stimulate further research, and call attention to some of the fields into which it might advantageously be directed.

ASA C. CHANDLER

Reactions with Drug Therapy, by HARRY L. ALEXANDER, M.D., W.B. Emeritus Professor of Clinical Medicine, George Washington University Medical School; formerly Editor of the Journal of Allergy. Saunders Co., Philadelphia, 1955, 301 pages with index.

This monograph by a former editor of the *Journal of Allergy* is well timed and very properly oriented. No other concise source of such material, as covered in this book, is available. While there is extensive literature on *qualitative* untoward drug reactions, the common offenders, especially those causing dermatoses, are probably limited to not more than 1,000 agents. Despite the opinion that practically any drug is allergenic, a comparatively small number of common lesions account for most reactions.

Thus, after an appropriate introduction, the author has dealt with the subject in a simplified fashion and in a very direct manner. The second chapter is concerned with the mechanisms of "hypersensitivity," in contrast to expected pharmacologic responses to drugs.

The third chapter deals with dermatologic manifestations, and falls into the classification usually preferred by dermatologists. Chapter IV deals with systemic patterns, including blood dyscrasias, serum sickness, hepatitis, and so on. Chapters V and XVIII deal with drug groups, such as antibiotics, other anti-infectious agents, serums, vitamins, and vaccines, to mention those of use in tropical medicine.

While limited sections hold great interest for the practitioner in the tropics, the monograph probably will have larger acceptance by practicing physicians in more general practice.

HAMILTON H. ANDERSON

Antisera, Toxoids, Vaccines and Tuberculins in Prophylaxis and Treatment, by H. J. PARISH, M.D., F.R.C.P.E., D.P.H. Clinical Research Director, Wellcome Foundation Ltd.; formerly Bacteriologist, Wellcome Research Laboratories. 3rd edition: 227 pages, illustrated, with 19 in color. Baltimore, Williams and Wilkins Co. for the Wellcome Foundation Ltd. 1954. Price $5.00.

The third edition of this little book, first published in 1948, is a worthy omen. The book is improving. Originally called *Bacterial and Virus Diseases*, a misnomer, the present title indicates the topic which has been the goal from the start, a discussion of specific biologic products: vaccines, serums, and skin test products. This is the best organized of the three editions and is the first to show signs of orderly cohesion.

The essentials of a biologic product other than the prinicples are: (1) the manufacture, (2) the bases of true or analogous standardization and tests for safety, (3) the rationale of use, (4) the application, and (5) a critical evaluation. Some of this is given for most of the biologic products, written with the usual British clarity and presented on the whole conservatively. Since biologic products are given remarkably little analytic consideration anywhere, even in medical meetings, the facts included herein are all valuable and, within the limits of facts, authoritative. There is no better book on the topic available, there is little to misunderstand and much to inform, and the book avoids the misleading elements so popular in this field.

That the book has a path ahead of it is more, rather than less, evident in this edition, since it now approaches an orderliness not before so apparent. The outline of products and the principles needs clarification and broader and more solid discussion. Certainly the first chapters should deal with vaccines or Parish's "prophylactics," not with serums. The products need logical sequence and development. Above all, strength is needed in standardization and evaluation in use, including dangers and reactions, for on these hinge the principles and applications of these products. The volume is not a guide to physicians, a handbook for producers, a basic text for students, nor even quite a hybrid of the three, but it has now reached a point wherein it could become such. Certainly in a world in which emotionalism can be turned loose as it has been in the realm of vaccines, from the days of stoning Cotton Mather and Zabdiel Boylston to the reversal in which experts have stoned the public with dubious products, the obligation to present a dispassionate appraisal of facts, for which Parish has all along had obvious training and talent, has been needed. Once this is done, good cash can well be saved by dropping most of the color prints. The sentimental attractions of a color print of such as the Schick Test are grossly offset by the dissimilarities between the print and the reactions which occur.

MAX S. MARSHALL

Introduction to Parasitology with Special Reference to the Parasites of Man, by ASA C. CHANDLER. (9th ed.) John Wiley & Sons, Inc., N. Y., Chapman & Hall, Ltd., London, 1955. 799 pp. Price: $8.50.

This latest edition of Chandler's "Introduction to Parasitology" has all the excellent qualities of its predecessors. It is so enlarged that the title no longer connotes the wide coverage of the subject. In fact, the clinical and public health features take the contents well beyond the category of an introductory textbook designed for use in undergraduate curricula where the subject is usually introduced. However, clinical discussions, as pointed out in the preface, are not dealt with sufficiently to satisfy the medical student—these are left for the professor to fill in. Chemotherapy and the newer treatment techniques are well handled, but at times such information is hidden. For example, the recent use of ACTH in trichiniasis is omitted in the discussion on trichiniasis but is briefly mentioned in "Introduction to the Worms." Descriptions under the headings of pathology deal, for the most part with symptomatology rather than with pathology. Although veterinary parasitology is given secondary consideration, veterinary students will find this book useful as a reference.

The book is well written in the interesting style characteristic of Dr. Chandler. Like

other textbooks, "Introduction to Parasitology" has its oversights and misstatements but anyone with any interest in parasitology will find it highly informative reading—it is next best to a talk with Dr. Chandler.

DONALD L. AUGUSTINE

Biochemistry and Physiology of Protozoa, Volume II, by S. H. HUTNER, Haskins Laboratories, New York, New York; and ANDRÉ LWOFF, Dept. of Microbial Physiology, Pasteur Institute, Paris, France, Editors. 388 pages, illustrated. New York: Academic Press Inc. 1955. Price $9.00.

This volume and its predecessor (Vol. I, 1951) provide a wealth of fundamental information concerning the mechanism of one-celled organisms, including both free-living and parasitic species. For biochemists and physiologists an evaluation of the contents probably should be prepared by a biochemist; for protozoölogists, by a comparative protozoölogist, and for workers in medical parasitology and tropical medicine, by one who is familiar with this area of knowledge.

First of all the reviewer is impressed with the choice of contributors, and secondly with the breadth and stature of volume II, based not only on a vast amount of new data which have accumulated during the short interval between publication of the two volumes but likewise on increasing ability to fit the information into certain patterns common to the Protozoa and other unicellular organisms. Much of the success which has been achieved is due to a wider coverage of subjects, and to new points of view. Yet there is considerable diversity of opinion among the contributors to the present volume with respect to the stage of biochemical knowledge which has thus far been attained. In the introductory chapter the senior author is perhaps overly enthusiastic in his conviction that protozoa should be used "as tools for analyzing morphogenesis in metazoa" because "for protists, thanks to comparative biochemistry, there is a stock of well-organized information on 'cell metabolism,'" and in his belief that axenic cultures of certain free-living species of protozoa will provide clues to their phylogenetic relationship. In contrast, Maurice Sussman, who writes on "Developmental Physiology of the Ameboid Slime Molds," remarks: "The most general conclusion that one can reach at this stage of the investigation into slime mold development is that no general conclusions are as yet possible;" and W. J. van Wagtendonk, in discussing "Encystment and Excystment of Protozoa," states that both are complicated processes which "are little understood, chiefly because in most instances rigorously defined conditions have not been established."

Although of scholarly content, the chapters dealing exclusively with free-living species of protozoa will not be commented on here. Chapters of particular interest to readers of this journal are "The Chemotherapy of Malaria, Piroplasmosis, Trypanosomiasis and Leishmaniasis," by L. G. Goodwin and I. M. Rollo, and "Comparative Studies on Amebae and Amebicides," by William Balamuth and Paul E. Thompson. In introducing the former chapter the authors emphasize the basis for their presentation, namely that "parasite organisms make use of enzyme systems similar to those of the host and that the ability of a drug to kill the one and spare the other depends upon small differences in detail of the metabolic process;" and, later, that "the general pattern of an antimetabolite which may be of value as a chemotherapeutic substance may sometimes be forecast from the structure of the metabolite it is wished to antagonize." These workers then take up for each of their main disease groups the rationale and methods of approach, and the antimetabolite efficiency of the newer drugs, as well as potentiation, inhibition, and developing drug resistance.

In the chapter on Amebae and Amebicides an introductory statement is made that in spite of apparent similarity the amebas are strikingly diversified, living in the sea, fresh water, soil, feces, and as parasites of many groups of hosts, either in the lumen or tissues, and varying a thousand fold in size. While monoxenic cultivation may usually be achieved, only free-living forms have thus far been grown for any length of time without a living microbial associate. Metabolic studies on the parasitic amebas of man have been confined

primarily to attempts to cultivate these species, and particularly *Entamoeba histolytica*, in quasi-synthetic media, to define the pathways of carbohydrate and protein metabolism, and to discover their enzyme systems. The evaluation of amebicidal action of drugs against *E. histolytica* is "complicated by the difficulty of distinguishing between direct effects upon the amebae and the indirect effects resulting from inhibition" of associated micro-organisms. Methods employed for circumventing this obstacle are tabulated. *In vitro* results are compared with those obtained in appropriate experimental animals, with extensive tabular summaries of the drugs found to effect direct and indirect action, arranged according to the structural formulas of the drugs. It is concluded that there is very little convincing evidence as to the mode of action of any antiamebic drug.

Each chapter in this volume is well documented with source references. The Author Index includes both text and reference page citations. In the Subject Index there is a preponderance of biochemical terms but common and scientific names of organisms are also well represented. All in all, this volume is a distinguished successor to Volume I of the series. Perhaps in another four or five years accumulated new information will provide a demand for a third volume.

ERNEST CARROLL FAUST

Entomology (Medical & Veterinary) Including Insecticides and Insect and Rat Control, by D. N. ROY, M.D., D.T.M., D.Sc., Director and Professor of Medical Entomology, School of Tropical Medicine, Calcutta (Retd.), In-charge of the Department of Medical Entomology & Biology, Indian Institute for Medical Research, Calcutta; and A. W. A. BROWN, M.B.E., Ph.D., Professor and Head of the Department of Zoology, The University of Western Ontario, London, Canada. 2nd edition: 413 pp., illustrated. Calcutta: Excelsior Press, 1954. Price $s.30, 45s., $8.

The present volume is essentially two independently written books. The first part, written by Dr. Roy, deals with medical and veterinary entomology and the control of arthropods. The second part, written by Dr. Brown, treats of insect toxicology, the application of insecticides and the chemical control of arthropods; a few pages are devoted to the chemical control of rodents.

The senior author frequently employs inaccurate scientific names and synonyms for micro-organisms, arthropods and reservoir hosts. Furthermore, he is not consistent in the usage of scientific names of arthropods. Rather often, grossly inaccurate and contradictory statements are made; for example, "they (*Amblyomma cajennense*) are very abundant in Texas . . . . This tick does not extend its range north of Mexican border" and "*O. coriaceus* recorded in Mexico. It is doubtful if it bites man." The typographical errors are abnormally numerous. The arthropods of medical and veterinary importance in India are somewhat over-emphasized at the expense of those of equal or greater importance elsewhere.

The discussion of Indian anophelines is excellent. The section on malaria control is brief, but good. The keys to adult female and larval *Anopheles* of India are very helpful and well illustrated. Good emphasis is placed upon mosquito breeding places. The chapters dealing with the Culicidae are the best in the part of the book covering arthropods. The description of techniques should be very helpful to the field worker who lacks advanced professional training.

The section of the book written by Dr. Brown is outstandingly excellent. He has accomplished successfully the difficult task of presenting briefly, but entirely adequately, the basic principles of insect toxicology, the fundamentals of the chemistry of insecticides, the methods of and descriptions of equipment for the application of insecticides, and the chemical control of arthropods of medical and veterinary importance.

The book should, in the opinion of the reviewer, be regarded as a reference book rather than as a textbook.

M. A. STEWART

The Therapy of Skin Tuberculosis, Publication No. 229, American Lecture Series, a Monograph in the Bannerstone Divn. of American Lectures in Dermatology, by GUSTAV RIEHL, M.D., Professor of Dermatology, University of Vienna; and OSWALD KOPF, M.D., Dept. of Dermatology, Wilheiminen Hospital, Vienna; translated and revised by ERNEST A. STRAKOSCH, M.D., Ph.D., Director, Dept. of Dermatology, Presbyterian Hospital, Denver, Colorado. 247 pages, illustrated. Springfield, Illinois: Charles C. Thomas. 1955. Price $6.75.

This book represents an extensive experience in the management of cutaneous tuberculosis, a group of diseases comparatively rare in this country. The authors discuss the results of Vitamin D therapy in 700 cases of tuberculosis of the skin, the majority of which represent lupus vulgaris. There are excellent before and after photographic illustrations as well as histologic follow-up. Various treatment techniques, contraindications and toxic manifestations of the valuable chemotherapeutic agent are adequately covered. A list of dosage schedules employed by other workers is documented. There are good chapters on antibiotic therapy with streptomycin and chemotherapy with the thiosemicarbozones and para-aminosalicylic acid. There is an excellent discussion of the use of isonicotinic acid hydrazide (INH) in cutaneous tuberculosis. The authors emphasize the superiority of this agent over all previous individual therapies.

A noteworthy error of omission is the absence anywhere in the book of a working classification of the various types of cutaneous tuberculosis. In the reviewer's opinion the book lacks organization and cohesiveness. In the attempt to be all inclusive with respect to historic detail, the reader is confused with a multitude of outmoded therapeutic modalities. A final summarizing chapter emphasizing modern optimum therapy would seem indicated. The inclusion of a case of "chronic joint rheumatism" treated with Vitamin D, as part of a series of 159 patients of lupus vulgaris, leads one to reflect on the validity of the statistics. The translation from the German fails in many places to effect grammatically correct English, making for tedious reading. There are numerous errors in spelling.

There is a definite need for a publication on this subject for the student and practitioner of dermatology. This book is of value to all interested in cutaneous tuberculosis because of the large experience of the authors. It leaves much to be desired from the standpoint of classification, concisemess, editorial organization, and clarity.

GEORGE H. KOSTANT

The Black Flies (Diptera, Simuliidae) of Guatemala and Their Role as Vectors of Onchocerciasis, by HERBERT T. DALMAT, Laboratory of Tropical Diseases, National Institutes of Health. Smithsonian Miscellaneous Collections, Vol. 125, No. 1 (Publication 4172) pp. I–VII, 1–452, Frontispiece, 43 plates, 13 text figures, 15 maps, 36 tables. Washington, D. C. April 5, 1955.

This volume brings together the results of nearly seven years of intensive work on the black flies of Guatemala. While the bulk of the book is devoted to the more strictly entomological aspects of the subject, the whole problem of *Onchocerca* transmission is well reviewed, including the work done in Guatemala.

The book is planned to give as broad a picture as possible of the environment in which onchocerciasis occurs in Guatemala, so that the physical factors, plant and animal associations, and human inhabitants are all discussed in some detail, and their bearing on the transmission of the disease emphasized. The species of black flies occurring in Guatemala are treated in great detail, with keys to adults of both sexes, pupae, and larvae. Male and female genitalia of both sexes, pupal respiratory filaments, cocoons and larval structures are also completely illustrated, so that there should be no difficulty in determining any Guatemalan blackfly in the foreseeable future. The natural history of all the species is discussed, with special emphasis on habitat and food preferences, while the geographical distribution in Guatemala is given in great detail, illustrated with maps. Three species believed of major importance as vectors of onchocerciasis were studied with especial care, and it is difficult to suggest any aspect of their lives which was neglected. There is a very

full bibliography and three appendices detailing the plants and animals of the main onchocerciasis zone and a summary of the important ecological factors affecting the three chief vector species, presented as a series of tables.

The work is a veritable mine of information and stands as a shining example of what can be accomplished by a long-term, consistent program. It should be read by every medical entomologist, if only as an example of what can be done.

G. B. FAIRCHILD

Ion Exchange and Adsorption Agents in Medicine, by GUSTAV J. MARTIN, National Drug Co., Philadelphia. Little, Brown and Co., Boston, 1955, Price $7.50.

The author has had a personal interest in ion exchange resins for more than ten years. His major contention is that all chronic degenerative diseases have as an important component in their etiology, the absorption from the intestine of small quantities of toxic chemicals.

The present monograph is an attempt to substantiate the author's premise. However, while it may have some validity in part, such an hypothesis would appear to be an oversimplification of the multi-dimensional aspects of degenerative diseases.

HAMILTON H. ANDERSON

Poliomyelitis, WHO Monograph Series No, 26, by ROBERT DEBRE and 16 other contributors, 408 pp., illustrated. Geneva: Palais des Nations: World Health Organization, distributed in the United States by International Documents Service, Columbia University Press, 2960 Broadway, New York 27, New York, 1955. Price $8.00, £2, Sw.fr. 24, cloth.

This volume, prepared under the aegis of the World Health Organization by 17 authorities on poliomyelitis, represents an attempt to summarize concisely our present knowledge of this disease. The phases covered include epidemiology, clinical aspects, virology, immunology, and control.

The three chapters in the section on epidemiology were prepared by Dr. John R. Paul, Dr. James Gear and Drs. M. J. Freyche and J. Nielsen. Freyche and Nielsen discuss, on a world-wide basis, the incidence of poliomyelitis since 1920 and point out that the epidemicity of the disease appears constantly to be increasing and to be related with advances in hygiene and elevation of living standards. Paul and Gear give a terse but excellent summary of the epidemiology of poliomyelitis in general and present evidence to show that the most significant difference between the occurrence of poliomyelitis in well developed areas of the world and those less developed is not primarily a difference in incidence but in the distribution of cases in the various age groups. Those interested in the epidemiology of poliomyelitis will find these chapters highly illuminating.

On the clinical aspects, there is a chapter by Drs. Debre and Thieffry on the symptomatology and diagnosis of poliomyelitis. Dr. W. R. Russell discusses the management of acute poliomyelitis, and Dr. H. C. A. Lassen gives a comprehensive review of the management of respiratory and bulbar paralysis.

In the section on virology, Dr. Sven Gard describes some of the physical and chemical properties of the poliomyelitis viruses. Dr. A. J. Rhodes and his associates review the role of the Virus Laboratory in the diagnosis of poliomyelitis and outline the methods and techniques which are utilized, placing special emphasis on tissue culture methods. This is an excellent chapter which is recommended not only to the laboratory worker, but also to the clinician and epidemiologist, since it conveys clearly the intricacies and pitfalls of laboratory diagnostic work, as well as the types of materials which should be submitted for examination and how they should be handled. Dr. Enders discusses tissue culture methods and reveals the considerable advances which have been made in the few years since he and his associates first cultivated the poliomyelitis viruses in human tissue.

Dr. Hammon takes up the use of gamma globulin. And Dr. Sabin discusses immunity in poliomyelitis with special reference to vaccination, reviews briefly human and animal

tests with killed virus vaccines and presents the results of his own studies on avirulent variants of the poliomyelitis viruses. Dr. Koprowski summarizes his investigations on the immunization of man with living poliomyelitis virus and presents evidence that an effective and safe vaccine comprised of living attenuated viruses is an attainable objective. Those interested are referred to his chapter which contains a great deal of material in tabular form.

The final chapter in the book is by Dr. A. M. M. Payne, who takes up public health measures in the control of poliomyelitis.

The book is recommended not only to those actively working in poliomyelitis, but also to those who may have only an academic interest in the subject.

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HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Black Vine Weevil, Cabbage Maggot, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevils, Cotton Thrips, Cowpea Curculio, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrip, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Serpentine Leaf Miner, Spittle Bug, Strawberry Root Weevil, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Turnip Maggot, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ... and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevils, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

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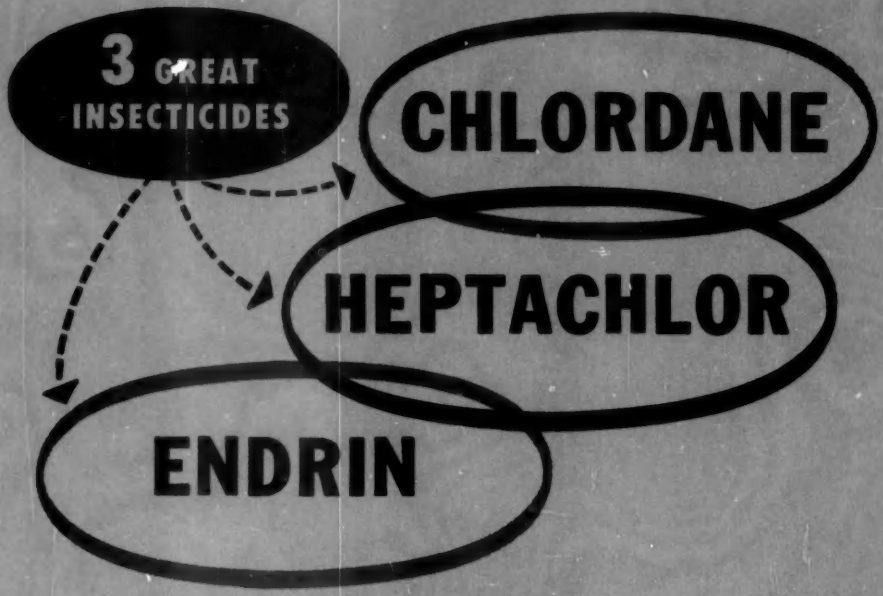
EDWIN H. LENNETTE, M.D.



CONTROL THESE INSECTS

CHLORDANE: Ants, Armyworm, Blister Beetles, Boxelder Bug, Brown Dog Tick, Cabbage Maggot, Carpet Beetles, Cattle Lice, Chiggers, Chinch Bug, Cockroaches, Crickets, Cutworms, Darkling Beetles, Dog Mange, Earwigs, Fleas, Flies, Grasshoppers, Household Spiders, Japanese Beetle Larvae, Lawn Moths, Lygus Bugs, Mole Crickets, Mosquitoes, Onion Maggot, Onion Thrip, Plum Curculio, Sarcoptic Mange, Seed Corn Maggot, Sheep Ked, Silverfish, Sod Webworms, Southern Corn Rootworm, Strawberry Crown Borer, Strawberry Weevils, Sweet Clover Weevil, Tarnished Plant Bug, Termites, Ticks, Wasps, White Grubs, Wireworms ... and many others.

HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Black Vine Weevil, Cabbage Maggot, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevils, Cotton Thrips, Cowpea Curculio, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrip, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Serpentine Leaf Miner, Spittle Bug, Strawberry Root Weevil, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Turnip Maggot, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ... and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevils, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

WRITE FOR FULL PARTICULARS

VELSICOL CHEMICAL CORPORATION VELSICOL

General Offices and Laboratories, 330 East Grand Avenue, Chicago 11, Illinois

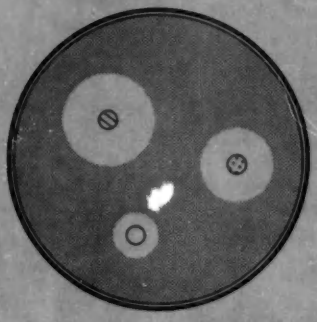
REPRESENTATIVES IN PRINCIPAL CITIES

DIFCO

Select ANTIMICROBIAL AGENTS *with* BACTO-SENSITIVITY DISKS

7 years' satisfactory clinical response to therapeutic agents selected with Bacto-Sensitivity Disks has demonstrated their *accuracy, dependability* and *rapidity.*

ANTIBIOTICS



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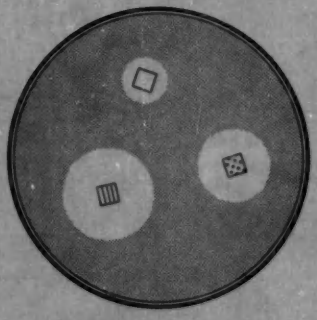

OTHER AGENTS

SPECIFY BACTO-SENSITIVITY DISKS *to assure*

- Maximum freedom of selection of therapeutic agents
- Broadest selection of concentrations
- Accurate readings without cross reaction
- Packaging for convenience of laboratory workers
- Greatest economy

Bacto-Sensitivity Disks are sterile and prepared in three concentrations of each therapeutic agent making it possible to determine relative sensitivity of the organism under investigation—*very sensitive, sensitive, slightly sensitive* or *resistant.*

Bacto-Sensitivity Disks are especially valuable in selecting the therapeutic agents effective against persistent infections refractory to primary therapy.

AVAILABLE ON REQUEST

- BOOKLET 146 *discusses the new, improved and expanded series of Bacto-Sensitivity Disks*
- NEW REPORT FORM *to record microbial sensitivity*

DIFCO LABORATORIES

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